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An International Journal of Genetics

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EARLY DAYS OF GENETICS *

R. C. PUNNETT

Received 28.x.49

MR CHAIRMAN, LADIES AND GENTLEMEN, may I first of all take this opportunity of thanking you for the high honour you did me in recently electing me an honorary member of this Society. Such honours, I regret to say, are always received with a certain regret because they almost invariably happen when one grows old and when one's senses are becoming dimmed, and for this reason, perhaps, I may seem to you considerably out of date. In reading a good deal of literature in the past few weeks I have frequently come across the word neo-Mendelism. Well, I suppose therefore that you are all neo-Mendelists. I do not know, I must confess, speaking as an ur-Mendelian, what it means, because the situation to-day seems to me to be the natural and logical outcome of what happened in 1900. So if I seem to you like a labyrinthodont addressing an assembly of Houyhnhnms you must forgive me.

It is related of Gregor Mendel, aware of his supreme discovery and of its neglect by the biologists of his day, that he uttered the words " Meine Zeit wird schon kommen." And I incline to think that he, who fought so strenuously in the Ultramontane dispute, would have felt no repugnance to the circumstance that his " Zeit " was also born in controversy. " Nur der Streit enthalt die Wahrheit " once wrote a distinguished German morphologist, and I feel that Mendel would have endorsed that saying. For the science of genetics was born in controversy and the manner in which this came about will take us back into the latter years of the past century.

Darwin had conquered in 1859 and for a generation the orthodox regarded the process of evolution as brought about by the action of natural selection on a continuously varying material. Natural selection, variation and heredity were the key words, though what were the limits of the two latter no one bothered much to enquire. It was the age of morphology, and the morphologist drew freely on the blank cheque which Darwin had provided. It was Francis Galton who first attempted to bring precision to these two key words by proposing the " Law of Ancestral Heredity," a " Law," as I have elsewhere pointed out, which had already been adumbrated by William Wollaston in 1722. The " Law " postulated a definite degree of resemblance, statistically measurable, in any familial relationship.

* An address delivered at the Hundredth Meeting of The Genetical Society, Cambridge, 30th June 1949.

If these values, as worked out from collected data, agreed closely with those postulated by the "Law of Ancestral Heredity" they would offer strong presumptive evidence in favour of the orthodox Darwinian position that natural selection worked by the accumulation of small continuous variations. So came into being the School of Biometry which, as Karl Pearson remarked, would eventually reduce biology to a branch of mathematics. This new line of enquiry was enthusiastically taken up by Weldon, then Linacre Professor at Oxford and one of the leading biologists at that time. On the mathematical side he had the support of Karl Pearson with whom he subsequently founded *Biometrika*, and with whose help were developed the correlation tables for working out the values of parental and fraternal resemblances. The great assumption made throughout was continuity of variation.

We may now turn to more personal matters. Weldon and Bateson were up at St John's * together and at that time were close friends. Weldon, who was senior to Bateson, was a dominant personality, and in referring to those days Bateson said that he was often made to feel like Weldon's bottle-washer. Both were convinced that morphology had played its part and that for further progress an attack must be made on the problem of species. It was here that their paths diverged. Weldon, as I have said, developed Galton's views with their assumption of continuity in variation. Bateson, however, was not convinced. He was more of a naturalist than Weldon and could not get away from the specificity of species. I fancy that his thoughts turned in the direction they were to take through his friendship with Professor W. K. Brooks when he was working at Woods Hole on the development of *Balanoglossus*. His Wanderjahr on the Russian steppe was an attempt to gain evidence of evolutionary change through a change in the environment. On his return he decided that the study of variation itself offered the best line of attack on evolutionary problems. As he gathered in material from all sources—from stock breeders, fanciers, horticulturists and others, he became more and more convinced of the frequency of discontinuity in variation, and eventually published his views with his collection of facts in the *Materials for the Study of Variation* in 1894.

Bateson's book was a direct challenge to the orthodox view and Weldon accepted it as such. Thenceforward battle was joined and the first clash came in 1895 over the origin of the cultivated *Cineraria*. Orthodoxy held that these cultivated forms had come from a wild species through the accumulation of small variations in the hands of the horticulturist working towards the ideal he had in view. Bateson protested that the many varieties in cultivation had come through hybridisation between several recognised species, and in support of his view he brought forward evidence from experiments by Mr Lynch, then Curator of the Botanic Garden at Cambridge. But he fought a lone hand for, in the controversy that ensued, Weldon

* St John's College, Cambridge.

was supported by such botanists of note as Botting-Hemsley and Thistleton-Dyer. Convinced, however, of discontinuity in variation, Bateson now decided to devote himself to experimental breeding with a view to discovering how these discontinuous variations behaved in the hereditary process. In this work he was joined by his first colleague, Miss E. R. Saunders, to whom this Society owes much. Crossing experiments were started with sweet peas, poultry and stocks, the last being Miss Saunders' province.

The aim of the new work was outlined in an address given to the International Congress on Hybridisation convened by the Royal Horticultural Society in 1899. It reveals how near Bateson's aims and methods were to those of Mendel. Then came the sudden revelation in 1900, when in the train on the way to deliver a lecture before the Royal Horticultural Society. How he revised his lecture on the journey and brought to this country the first news of Mendel's discovery you may read in Mrs Bateson's life of her husband. Instant and deep as was Bateson's appreciation of what Mendel had done there was another who also understood what it might mean. For Weldon was sufficiently alert to realise that discontinuity in heredity meant an end to that continuity in variation upon which the Biometrical School was founded. So the first step in the Mendelian controversy proper was provided by Weldon's belittlement of Mendel's work in the first volume of *Biometrika*.

Bateson's rejoinder was delayed. For on reading Mendel's paper he at once set to work to repeat the experiments. It was characteristic of him that when the rejoinder came in 1902 it took the form of a small book entitled *A Defence of Mendel's Principles of Heredity*. The gloves were off and Weldon was not spared. He retaliated with a further article in *Biometrika* in 1903. To this Bateson replied in a letter to *Nature* which, however, was never published since the editor stated that he was " not prepared to continue the discussion on Mendel's Principles and therefore returns herewith the papers recently sent him by Mr Bateson." Why, you may ask, did not Bateson send his reply to *Biometrika*, the venue chosen by Weldon for his criticism ? The answer is that the pages of *Biometrika* were also closed to him. In 1901 Pearson published a long paper in the Philosophical Transactions on what he termed " Homotyposis." As with all Royal Society papers it was submitted to referees of whom Bateson was one. His opinion was by no means favourable and he felt it incumbent on himself to justify it in a short paper in the Proceedings. This drew forth an answer from Pearson in *Biometrika* 1902. Bateson did not consider this satisfactory and submitted a further consideration of the matter. This the editors of *Biometrika* refused to accept for publication, so Bateson finally had it privately printed by the Cambridge University Press, using the format of *Biometrika*.

It was a difficult time for struggling geneticists when the leading journals refused to publish their contributions to knowledge, and we

had to get along as best we could with the more friendly aid of the Cambridge Philosophical Society and the Reports to the Evolution Committee of the Royal Society. And since most of the earlier genetical work was published in these Reports, I may digress for a moment to consider how this came about. At the instigation of Francis Galton the Royal Society in 1894 appointed a Committee for conducting statistical enquiries into the measurable characters of plants and animals. In 1897 Bateson was invited to become a member but, recognising its biometrical import, he declined. Later on, when the Committee was reconstituted as the Evolution Committee, he joined and eventually became its secretary. Weldon resigned and with Pearson later founded *Biometrika*. The Evolution Committee proved of great service both by providing small grants towards the experiments, and by affording a means of publication.

Meanwhile Weldon dropped the pea and started a new line of attack with mice, instigating a young pupil of his, A. D. Darbishire, to do the work under his direction. Darbishire was a pleasant young man but rather woolly and very much under Weldon's thumb. One of Weldon's complaints against Mendelism was what he called "Neglect of Ancestry," and the mouse work was intended to bring this out. A cross was made between albino and agouti and the F_2 generation gave of course 25 per cent. of albinos. Now one kept on breeding from the agoutis of the F_2 and subsequent generations *en masse*. At each generation the proportion of albinos diminished until eventually there were none at all. Clearly a proof of the influence of ancestry so shamefully neglected by the Mendelians. The fallacy of course was that no distinction was made between homozygous and heterozygous agoutis, and since the former class inevitably came more and more to preponderate owing to the method used the proportion of albinos would inevitably diminish. The experimental results were not presented in a form such as to satisfy a critical mind like Bateson's and a controversy ensued which was fought out in the pages of *Biometrika* and *Nature* until the latter journal refused to publish anything more from Bateson.

But before the mouse controversy reached its end a dramatic change took place. And at this point I may perhaps be excused if I indicate how I came into the story. I had become interested in sex determination and was proposing to start some experimental breeding of mice to find out whether it could be influenced by nutritional or other factors. So I asked Bateson whether, in the designing of my experiments, I could help to provide data for colour inheritance. The outcome was the charming letter of December 1903, reproduced in Mrs Bateson's *Life*, and thenceforward I became a privileged member of the Grantchester household. At the time I joined him Bateson's disciples were few. Besides his old colleague, Miss Saunders, his sister-in-law, Florence Durham, had begun to breed mice and canaries, while C. C. Hurst was engaged near Leicester

with poultry and rabbits. Biffen also had started those experiments with wheat that were to bring rust resistance into the category of Mendelian characters.

You will note that the little band round Bateson in 1904 were all seniors, that is of M.A. standing, and that they gained their livelihood from pursuits other than genetics. Miss Saunders and Miss Durham lectured to Newnham on botany and physiology respectively, Biffen gave lectures on mycology, and I myself was a demonstrator of zoology ; Hurst was a professional horticulturist, while Bateson kept himself by looking after the kitchens of St John's. But Bateson knew very well that if this new branch of enquiry was to live and expand it must capture young men and women, and the opportunity came with his appointment as deputy to the Professor of zoology in 1899. The post was not as important as it sounds for at that time the position in the department was a curious one. Professor Newton, a great ornithologist and a great gentleman, gave a few sparsely-attended lectures, while the large department, with a couple of hundred students or more, was run by Adam Sedgwick, Reader in morphology, assisted by a staff of lecturers and demonstrators. The teaching was entirely morphological and no one felt any interest in the issues which Bateson had raised. Newton's failing health gave Bateson his opportunity of reaching the younger students, with the result that Lock, Doncaster, Gregory, Miss Wheldale, Miss Sollas, Miss Marryat and others were soon brought into the fold.

However, let us hark back to 1904, when my personal recollections start. The set-up was primitive for money was scarce. The poultry occupied a small paddock split up into about two dozen little pens. There were several incubators in a bedroom upstairs though this had soon to be given up since it was requisitioned for the little boys' governess. The chicks were reared in movable brooders along the garden paths. It was not a very satisfactory arrangement for, in a wind, one of them occasionally caught fire, and there was an end to *that* hatch. We had only oil, which meant much work in filling the various receptacles and cleaning the wicks. Every afternoon one of us went out collecting and marking the eggs from the various pens. We had no trap nests but soon became adepts at spotting the egg laid by each particular hen. All birds of course were numbered, and Bateson had devised an ingenious little brass label which clipped round the leg of the newly-hatched chick. These were labelled consecutively with the year on the other side, and each number corresponded to a page in the record book. When the copper ring became too tight for the swelling leg the label was cut off and attached to the wing, an operation we called " wiring." Though rather a tedious operation it was always interesting since it provided speculation on various points. Evidence that we did not always agree could be found by the curious in the form of bets recorded throughout the pages of the notebooks. Moreover, in deciding what to keep, and what to

reject, we had to be all the time planning the programme for the coming year. Birds rejected were destined for the higgler, for even the small sums so received helped to defray the cost of the work.

Though we reared some hundreds of chicks each year the great majority of the eggs incubated were never allowed to hatch, for some of the characters on which we were working were sufficiently developed for determination at about the eighteenth day. So we had periodical " openings " which were recorded in a separate notebook known as the " Dead Book." On the day of an " opening " we adjourned to the outhouse in which the incubators were later kept, having previously collected Mrs Bateson to clerk for us, a function which she performed with the greatest efficiency and devotion. Having settled her in a chair at the trestle table with the " Dead Book " and a large bowl, Bateson took off his coat and produced his knife with the big, blunt blade, while I stood by with a pair of scissors. He then took up an egg, read off the numbers of the pen, the hen and date of laying, and after " Have you got that, Beatrice? " proceeded to stab and peel off the shell into the aforesaid bowl, and to call out the peculiarities of that particular embryo such as lt., nts., r.c., n.e., f.l., which was to be interpreted as " light down, no coloured ticks seen, rose comb, no extra toe, feathering on leg." After which the chick was handed to me, who slit it so as to expose the sex glands and give Mrs Bateson the sex to complete the entry. Altogether it was a messy job and " openings " were not much looked forward to.

Sweat peas were the other main line of enquiry. We grew some thousands each year and of course the garden at Merton House could not nearly accommodate such numbers. Mrs Bateson protested that vegetables were necessary to keep the household alive. But we managed to borrow a small plot from a neighbour, and the Agricultural Department very kindly allowed us the use of about an acre of rough arable on the University Farm. Only the precious first crosses and the few families we knew we should want for further breeding were privileged to occupy a garden site and to be cleaned and sticked by us. The rest were sown in drills, mainly on the University Farm which was then at Impington, beyond Histon. We arranged with a farm labourer to eradicate the worst weeds, otherwise they received little attention until the time came for " pulling." Then we set out on the four-mile ride for a long afternoon, Bateson with his wife in the trailer carrying the " Farm Book " and a microscope. Having made Mrs Bateson reasonably comfortable we proceeded to pull, family by family. One of us pulled the plant and sung out its characters (*e.g.* ppw., dk., ax.) and handed the plant to the other, who, with the microscope perched on some odd box picked up at the farm, determined the shape of the pollen. All duly logged by Mrs Bateson. In the garden, of course, we were busy, during the flowering period, emasculating, crossing and recording. This at times meant a lot of work especially after we had hit upon the 9 : 7 ratio and the interaction of factors, for we felt it

necessary to verify this by hundreds of crosses among F_2 whites, and this meant getting up at 6 a.m. and putting in a couple of hours before breakfast, before the anthers had a chance of bursting.

My initiation into these studies was a strenuous one and I was so busy picking up the threads of a new pattern of research that I never fully realised, until it was past, how important 1904 was to be. I had, of course, obtained some insight into the nature of the antagonism between Weldon and Bateson as we chatted in the course of our work. And I knew that Bateson was to be President of the zoology section at the coming meeting of the British Association. I had never attended a meeting of this body and had not thought of it as of any importance to the work we had in hand. Bateson, however, had realised that this was a great opportunity. It was constantly in his thoughts and he neglected nothing in order to make his own part a success. When the sweet pea season came on in June he used to take a chair and a little table out into a small copse at the back of the chicken pens, leaving the strictest orders that he was on no account to be disturbed by anyone. So each day when I arrived with the *Morning Post*—a paper of which Bateson approved since it contained the best account of art sales, and I don't think he read much else—I set to work cleaning, recording and labelling sweet peas while Bateson, in the seclusion of his copse, struggled with his address. But he knew that I was there and after a time would saunter out and invite discussion on some particular point. It was, of course, to ventilate his mind, and the only point wherein I was of any help was to draw his attention to Archbishop Parker's forbidden degrees of marriage at the end of the *Book of Common Prayer*.

Eventually, all was set for the great day—somewhere in August if I recollect right. To section D was allotted the large lecture room in the newly erected geological building, and the place was full. Bateson had his say, and how striking a say it was can be judged by anyone with access to a copy of Mrs Bateson's memoir. Hitherto he had been on the defence ; now he attacked, and such statements as "the imposing Correlation Table into which the biometrical Procrustes fits his arrays of unanalysed data is still no substitute for the common sieve of a trained judgment," admitted no doubt of the direct challenge. After the Presidential Address the first meeting of the section was devoted to papers and discussion on Mendelian matters. Bateson deployed his forces. The morning was given up to facts. Miss Saunders authoritatively announced her findings in the stocks. Hurst assured the audience of Mendelian phenomena in poultry, while I chimed in with the story of the fowl's combs.

We adjourned for lunch and on resuming found the room packed as tight as it could hold. Even the window sills were requisitioned. For word had gone round that there was going to be a fight. Probably other meetings were depleted—but after all the Association is British. Weldon spoke with voluminous and impassioned eloquence, beads of

sweat dripping from his face, and I cannot help recalling the admiring remark made by one young Oxford man to another as they sat just in front of me, " Clever beggar that—he hasn't got to stop and think." Bateson replied and there may have been other speakers, I have forgotten. But towards the end Pearson got up and the gist of his remarks was to propose a truce to controversy for three years, after which the protagonists might meet again for further discussion. On Pearson resuming his seat, the Chairman, the Rev. T. R. Stebbing, a mild and benevolent looking little figure for a great carcinologist, rose to conclude the discussion. In a preamble he deplored the feelings that had been aroused, and assured us that as a man of peace such controversy was little to his taste. We all began fidgeting at what promised to become a tame conclusion to so spirited a meeting, especially when he came to deal with Pearson's suggestion of a truce. But we need not have been anxious, for the Rev. Mr Stebbing had in him the makings of a first-rate impresario. " You have all heard," said he, " what Professor Pearson has suggested " (pause), and then with a sudden rise of voice, " But what I say is let them fight it out." And on that note the meeting ended. Bateson's generalship had won all along the line and thenceforth there was no danger of Mendelism being squelched out through apathy or ignorance.

But in spite of the success of the Cambridge meeting in getting Mendelism a hearing the older generation of biologists endorsed Weldon's hostility and the pens of Alfred Russell Wallace, Professor Poulton and Professor J. Arthur Thomson were soon engaged in attempting its belittlement. In this they were supported by *Nature*, though by now such hostility was of less account ; Mendelism had become news and the columns of secular periodicals were opened to us.

Weldon's next attack was directed at Hurst. Now Hurst was a tireless worker and full of ideas, but over-apt to find the 3 : 1 ratio in everything he touched. While valuing Hurst's enthusiasm for the cause Bateson was nevertheless mistrustful of his slickness, for he knew that his critical ability had not been sharpened by passing through the scientific mill. Hurst's acquaintance with hunting folk had suggested to him that chestnut was recessive to bay and brown. He collected data from the *Stud Book* and got Bateson to let him read his paper before the Royal Society. Knowing what was afoot Weldon had also consulted the *Stud Book*, and at the meeting he criticised Hurst severely by bringing forward cases in which chestnuts mated together gave bay or brown offspring. Bateson felt that Hurst had let him down, and he was intensely irritated when Hurst blandly assured Professor Weldon that he was mistaken and that these alleged exceptions were mere errors of entry. At the close of the meeting Bateson withdrew the paper, and together with Miss Durham we set out on foot for somewhere north of Oxford Street where we were due to dine with the Herringhams. It had happened that at this same meeting Bateson had read our communication on the 9 : 7 ratio in

the sweet pea and had illustrated it with a large hanging diagram. So through the length of Bond Street he marched, grim and silent, shouldering a 6 feet roll of paper with Miss Durham and myself trotting behind him in a state of mixed apprehension and amusement. Dinner that evening was a glum affair.

The Christmas vacation was devoted to an intensive study of the *Stud Book*. Most of Weldon's alleged exceptions turned out to be of little importance. A stillborn foal requires a return and it is of little matter to the equestrian world whether the groom chooses to call it a bay or a chestnut. Though most of the exceptions were of this nature there was a more serious one with which he had made great play. " Ben Battle " was the horse's name and though registered as a chestnut he had sired bays from chestnut mares. There seemed no getting over this until suddenly Hurst came to the rescue. He consulted a work called *Form at a Glance*, a work unknown to us but with a title expressive of its contents. And one day there came a welcome telegram, " Ben Battle never *ran* as a Chestnut." Though he could not have known it at the time, Hurst's bland assurance to Professor Weldon turned out to be right, and a month later Bateson recommunicated the paper which was then published in the Proceedings of the Royal Society.

At this period we knew that any flaw in our work would at once be pounced upon and subjected to bitter criticism. Not a bad thing really for it kept us on our toes and added a spice to the work. Then suddenly Weldon died ; the controversial stage fizzled out and genetics entered into its own.

Here I may mention an interesting advance in genetical theory which came about in an unusual way. In 1908 I gave an address to the Royal Society of Medicine on " Mendelian Heredity in Man." In the subsequent discussion I was asked why it was that, if brown eye were dominant to blue, the population was not becoming increasingly brown-eyed : yet there was no reason for supposing such to be the case. I could only answer that the heterozygous browns also contributed their quota of blues, and that somehow this must lead to equilibrium. On my return to Cambridge I at once sought out G. H. Hardy with whom I was then very friendly. For we had acted as joint secretaries to the Committee for the retention of Greek in the Previous Examination and we used to play cricket together. Knowing that Hardy had not the slightest interest in genetics I put my problem to him as a mathematical one. He replied that it was quite simple and soon handed to me the now well-known formula $pr = q^2$.* Naturally pleased at getting so neat and prompt an answer I promised him that it should be known as " Hardy's Law "—a promise fulfilled in the next edition of my *Mendelism*. Whether the battle of Waterloo was won on the playing fields of Eton is still, I

* Where p, $2q$ and r are the proportions of AA, Aa, and aa individuals in the population varying for the A-a difference.

gather, a matter for conjecture : certain it is, however, that " Hardy's Law " owed its genesis to a mutual interest in cricket.

For a few more years I worked with Bateson at Grantchester until, in 1910, he moved to Merton to take up the Directorship of the John Innes Institution, no longer to be frustrated by inadequate means. I look back upon those years as among the most pleasant in my life. No one more delightful to work with than Bateson could be imagined. Dominant personality as he was, he was never domineering. Only once can I recall his having lost his temper with me, and then he had every justification because I had imported a trio of Silky fowls without his knowledge. I was getting a little bored with the everlasting single, pea, rose and walnut combs and I had a hunch that the queer little Silky with its unusual comb might bring in something new. That it certainly did, and out of its crosses with the Brown Leghorn came the data which enabled us to establish the doctrine of sex-linkage. So I was forgiven. Another lucky venture was the preservation of the cretin sweet pea, a single plant of which turned up in a long row. It did not appeal to Bateson, who said, " Do throw away that ' monstrous thing '." But it intrigued me and I insisted on making some crosses with it. It turned up trumps for it led to our establishing that repulsion and coupling, hitherto regarded as distinct features, were all part of the same phenomenon, and by its means we were led to establish the principle of linkage.

I have sometimes been asked how it was that having got so far we managed to miss the tie-up of linkage phenomena with the chromosomes. The answer is Boveri. We were deeply impressed by his paper " On the Individuality of the Chromosomes " and felt that any tampering with them by way of breakage and recombination was forbidden. For to break the chromosome would be to break the rules. So it was left for Morgan and his colleagues to make use of Janssen's observations and by their brilliant work to link up genetics and cytology, thereby opening up a new era in these studies.

THE CYTOPLASM IN HEREDITY*

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I

DURING the past forty years many investigations have demonstrated that the cytoplasm has genetic functions. Correns (1909) and Baur (1909) led the way with their studies on variegation in higher plants. After observing cytoplasmic inheritance of a number of traits in mosses, von Wettstein (1927) set forth the concept of the plasmon, a system of genetic determiners in the cytoplasm. Plasmon-controlled traits have subsequently been reported in many other plants by a number of geneticists, foremost among whom are Michaelis and his co-workers on *Epilobium* (see P. and G. Michaelis, 1948, for literature). Comparable observations on higher animals are less common. L'Héritier (1948) and his colleagues have demonstrated cytoplasmic inheritance of sensitivity to CO_2 in *Drosophila*. Pigmentation in spotted guinea-pigs is interpreted by Billingham and Medawar (1948) to depend upon a cytoplasmic genetic particle. This spotting may be regarded as a manifestation of cytoplasmic inheritance on the cellular level. It is the commonest type of cytoplasmic inheritance in higher animals, probably occurring with regularity during their ontogeny, for different cell types taken from the same soma may maintain their diversities through countless cell-generations in tissue culture. Comparable cytoplasmic inheritance on the cellular level appears also in unicellular organisms, both in the form of hereditary variations within a clone and otherwise, as in *Paramecium* (Sonneborn, 1947*a*) and yeast (Ephrussi, L'Héritier and Hottinguer, 1949).

Cytoplasmic inheritance among both plants and animals, and in multicellular as well as unicellular organisms, is thus not an hypothesis, but a fact—one of the capital facts of biology. Hypothesis and opinion enter only when this fact is interpreted, when the physical bases of cytoplasmic inheritance and the mechanisms by which they operate are discussed. Even on the questions of physical basis and mechanism, there are a number of solidly established facts in one group of examples of cytoplasmic inheritance, but not in another group. To distinguish between the more and the less understood types of cytoplasmic

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inheritance, I shall separate my accounts and discussions of them into different sections of this paper. Section II includes the relatively well-understood type, section III the poorly understood type.

As will appear, this plan emphasises the existence of fundamental differences between different examples of cytoplasmic inheritance. These differences are not immediately apparent. Indeed, the different examples may at first seem to be similar in principle. I have learned this the hard way. In one of my first reports (Sonneborn, 1943) on cytoplasmic inheritance, it was pointed out that all nine known characters in several varieties of *Paramecium aurelia* showed superficially similar cytoplasmic inheritance and it was assumed the same sort of physical basis and mechanism was operating in all of them. The nine characters were of three kinds : (1) the production of antibiotic agents, *i.e.* the so-called " killer " traits ; (2) serotypes or antigenic types ; and (3) sex-like differences or mating types. One of the killer traits was analysed in detail in order to provide a model applicable to the other traits in *Paramecium* and possibly also to the traits in other organisms. This model does apply (so far as present information goes) to all of the killer traits and to many examples in other organisms ; but it failed to apply to the next kind of trait examined in *Paramecium*, the antigenic types. Apparently more than one model is needed to account for the various examples of cytoplasmic inheritance.

Our experiences with *Paramecium* indicate that the fundamental distinction among these diverse examples lies in the source of the specificity of the cytoplasmic basis of heredity. In one group, the nuclear genes are the source of specificity ; in another group, the cytoplasm itself controls its own specificity. The examples of the latter group seem to conform to a common model, the one developed in our study of the killer trait. In the following section an attempt is made to present and illustrate that common model, at the same time pointing out the gaps in knowledge, the uncertain aspects of the interpretation, and the lines along which may come further advances in knowledge and understanding.

II

(*a*) *Self-duplication and mutability*

The examples of cytoplasmic inheritance to be discussed in this section should be confined strictly to those in which the cytoplasmic basis of heredity is mutable, and therefore also self-reproducing. On these points the evidence is convincing and complete in but few cases. That a single model structure or particle is not only essential but sufficient, in the proper cellular environment, for the formation of more has been shown both for chloroplasts (Ternetz, 1912) and for kappa, the basis of the killer trait in *Paramecium* (Preer, 1948*a*). Those structures are clearly self-reproducing, as are many others to which we shall turn presently ; but mutability is known only for the plastids (see review by Rhoades, 1946), for kappa (Dippell, 1948,

and unpublished), and for sigma, the basis of sensitivity to CO_2 in *Drosophila* (Goldstein, 1949). The work on mutability will be illustrated by the study of kappa, but as the killer trait it controls will be referred to repeatedly in the ensuing discussion, I shall first digress enough to provide the necessary background (see Sonneborn, 1943, 1945, 1946, 1947*b*).

Killer strains of paramecia liberate into their culture fluid a poison, paramecin, to which they are resistant, but which kills in a characteristic way paramecia of other strains, known as " sensitives." These sensitives can be mated to the killers, however, because sensitives are unaffected by paramecin during conjugation. The nuclear processes of conjugation are such that the two mates of a pair acquire identical genotypes. They then separate and each produces a culture by repeated fissions. In spite of their identity in genotype (or at least in *nuclear* genotype), the cultures derived from the two mates of such a conjugant pair are diverse : one is a killer, the other is a sensitive culture. From these F_1 cultures, the F_2 obtained by selfing shows no segregation : all F_2 cultures derived by selfing among the F_1 killers are killers ; all F_2 cultures derived by selfing among the F_1 sensitives are sensitives. Hence, the difference between these killers and sensitives is due, not to a genic difference, but to a cytoplasmic difference.

This conclusion is confirmed by exceptional conjugations in which cytoplasm visibly flows from mate to mate across a connecting cytoplasmic bridge. After receiving cytoplasm from its killer mate, the sensitive conjugant produces a culture of killers. As before, no segregation occurs in the F_2. From the fact that killers can transmit the physical basis of the killer trait to sensitives across a cytoplasmic bridge, it was inferred that the killers possess a cytoplasmic genetic factor determining the killer trait. This is the factor known as kappa.

After some years of study of the properties of kappa by indirect methods, Preer (1948*b* and in press) was able to demonstrate that it is a microscopically visible particle. Fig. 2 shows the presence of a large number of kappa particles in the cytoplasm of a killer ; Fig. 1 shows their absence from a sensitive. Killers can be irreversibly transformed into hereditary sensitives by freeing them of kappa through exposure to high temperatures (Sonneborn, 1946), X-rays (Preer, 1948*b*), or nitrogen mustard (Geckler, 1949) and in other ways (Preer, 1946). Sensitives can be transformed back to hereditary killers by exposing them to concentrated suspensions of the broken-up bodies of killers (Sonneborn, 1948*a*). In this way, the sensitives acquire some of the kappa that had been liberated from the disintegrated killer animals. Kappa is thus a self-multiplying cytoplasmic particle which is formed only when some is already present.

The sensitives thus far described have the same genes as the killers and differ from them only in lacking kappa. There is, however, another kind of sensitive that not only lacks kappa, but carries different

genes from the killers. This kind of sensitive strain cannot be converted into a killer by exposing it to kappa released from the macerated bodies of killers. Further, when it is crossed to a killer, the F_2 generation derived by selfing among the killer F_1 clones, shows segregation of killers and sensitives in proportions expected for a single gene difference. Only when dominant gene *K* is present can kappa be maintained; in homozygotes for recessive *k* kappa cannot be maintained and any carried over from killers is quickly and irreversibly lost. Other loci also seem to be involved, but less strikingly, in the maintenance of kappa (Sonneborn, 1947*b*).

With this background, we may now turn to the study of mutability of kappa. Dippell (1948 and 1950) collected a number of mutations of the killer trait in stocks of variety 4 of *P. aurelia*. All the mutations were discovered in laboratory cultures of killers. Some of the mutants produce very little of the killing substance, paramecin, but remain resistant to its action. Other mutants produce a different kind of paramecin that kills sensitives with visibly different pre-death changes. When the type of killing changes, the pattern of resistance also changes, each kind of killer being resistant only to paramecin of the same type that it produces. Some mutants produce both kinds of paramecin and are resistant to both. Thorough breeding analysis demonstrated that these mutations involve no change in any gene affecting kappa or the killer trait: with respect to such genes, the original killer and its derived mutants are identical. In every case, the change could be traced to a change in kappa. This was most strikingly shown in the case of the mutants that manifested two kinds of killing. Dippell showed that these carried two kinds of kappa. By diluting kappa down to a single particle per cell and then letting it accumulate again, she was able to get "pure cultures" of the two kinds of kappa in different paramecia and their progeny. Thus kappa can mutate and the diverse mutant kappas can be maintained and multiplied in cells with the original genome.

Dippell's analysis proves that the specificity of kappa is inherent in kappa itself. With this the nuclear genes have nothing whatever to do: diverse kinds of kappa not only multiply true to type in diverse cells with the same genotype, but they even multiply together and maintain their specific diversities in one and the same cell and all of its cell progeny. The same sort of evidence for mutability of chloroplasts and sigma proves that they also are autosynthetic and mutable, that their specificities are determined by themselves, not by the nuclear genes.

While the full demonstration of both self-duplication and mutability has been given only for the three examples already mentioned, a large number of others probably belong in the same category. There is some evidence for both self-duplication and mutability of the plasmon in *Epilobium* (Michaelis, 1948) and for a number of so-called "viruses" that resemble genetic factors in their normal transmission

only by inheritance, not by infection (see Darlington, 1944 ; Haddow, 1944 ; Sonneborn, 1949). A number of cellular structures, although not known to be mutable, are visibly self-duplicating. This has been emphasised by Lwoff (1949) for the cinetosome of protozoa, the granule that lies at the base of each cilium and flagellum ; by Darlington (1949*a*) for the centrioles ; and by Sonneborn (1949) for the bacteroids and Rickettsias of insects, for mitochondria, for the basis of male sterility in a number of plants, and possibly for the microsomes. To this list should be added the anlage of the gullet of *Paramecium* (Sonneborn, 1947*a*, p. 273), and perhaps the physical basis of the " small-colony " mutant in yeast (Ephrussi, L'Héritier and Hottinguer, 1949). Essential structures of a cell, such as centrioles and cinetosomes, may not be capable of surviving a mutation with effects gross enough to be otherwise detected. Yet the visible self-duplication of these cytoplasmic structures shows they can scarcely lack genetic significance. Without evidence as to their mutability, however, the question of whether they or the nuclear genes control their specificities cannot be answered.

(*b*) *Size and organisation*

Attempts have been made (Darlington, 1944) to classify these cytoplasmic genetic factors on the basis of their size and structural complexity and to attribute different significances to the different classes. The chloroplast is certainly one of the largest, but it arises from a much smaller plastid which is of the same order of size as many other cytoplasmic genetic particles : mitochondria, centrioles, cinetosomes and kappa. Somewhat smaller are the microsomes which may also belong in the category of genetic particles. I have heard that sigma of *Drosophila* is believed to be still smaller. It seems that self-duplicating cytoplasmic structures may grade down from the giant chloroplasts to macromolecules not much, if at all, larger than the nuclear gene. If the size series is as continuous as now seems likely, the usefulness of interpretations based on different sizes may be questioned.

Size can hardly be considered apart from organisation and development. Because chloroplasts have the genic properties of duplication and mutability and because they develop from much smaller plastids, as giant salivary gland chromosomes were believed to develop from ordinary chromosomes, Imai (1937) postulated the plastogene as a genetic determinant within the chloroplast. He went even further and assumed that each chloroplast contains a single plastogene, because of the lack of evidence for plastids with mixed constitution.

The question thus arises as to whether there are grounds for believing that other visible self-duplicating bodies consist of smaller genetic units, as is presumed in the case of the chloroplast.

There are indeed some reasons for suspecting that kappa particles

may consist of smaller genetic units. Several lines of evidence suggest that some kappa mutants may arise in pairs by a breaking of the original kappa particle into two smaller particles. Three of Dippell's five killer mutants carried two different kinds of kappa when first detected. In at least two of these three mutants, both kinds of kappa differed in some way from the original. Since killer mutants have rarely been found, the existence of two mutant kappas in even one of the few killer mutants could hardly be a mere coincidence, unless there were some unknown reason why double mutants are much more likely to be detected than single mutants. It is further remarkable and suggestive that at least some of the mutant killers can carry about twice as many kappa particles as the original killer. Finally, Preer (in press) records that the kappa particles in killer stock *G* are about 0·4 micron long, while those in the derived mutant *G mi* are only about 0·2 to 0·3 micron long. In other stocks (not mutants of *G*), the size of kappa goes up to 0·8 micron.

These observations, taken together, suggest that kappa exists in multiples of some basic size unit. As to how small the basic unit may be, or how many such units are present in the smallest visible kappa particle, there is little on which to hazard a guess. Preer's report (1948*b*) that a single hit curve describes the X-ray inactivation of stock *G* kappa might be considered as weighing against the multiple unit organisation of kappa, but his later work (in press) raises doubt as to whether the inactivation of kappa is after all a single hit affair.

To the indications of a finer genetic structure in chloroplasts and kappa, may be added comparable indications for mitochondria and centrioles. As is well known, mitochondria have been reported to arise *de novo* (Forenbacher, 1911, and later observers), to increase and decrease in size from and to the limits of microscopic visibility (Lewitzky, 1924; Kassmann, 1926), and to divide (Horning, 1926, and others). Supernumerary centrioles also appear to arise *de novo* under some conditions and then to divide (Wilson, 1901, and later observers). These evidences of *de novo* origin of self-duplicating structures suggest that they may arise from and contain self-duplicating submicroscopic particles. Darlington (1949*a*) further infers the existence of genetic sub-units in the centromere, which is in some respects analogous to the centriole, from its capacity for misdivision.

If Imai's lead were followed, a special term would be coined to designate the hypothetical genetic sub-unit in each visible, self-duplicating structure. To "plastogene" would now be added "kappagene," "centriogene" and "mitochondriogene"; and eventually there would probably be proposed "cinetogene" and even, alas, "gulletogene"! To recite this incomplete list of awkward and cacophonous terms is in itself almost enough to reject them. If they are to have a name at all, let them have a common name emphasising their common cytoplasmic localisation and their common gene-like properties. The most appropriate term, it seems to me,

is Winkler's (1924) old term " plasmatic gene," stripped of its more recently acquired special limitations and condensed to its more familiar form " plasmagene."

But we must not be led astray by our prejudice in favour of sub-microscopic units of heredity. The existence of plasmagenic sub-units in any one of the self-duplicating, cytoplasmic structures has not yet been established, while the possession of the genic properties of self-duplication and mutability for the gross structure is fully demonstrated in some cases. It therefore seems to me unwise to limit the term plasmagene to the possibly non-existent sub-units and deny it to the structure as a whole. If and when the genic properties are in any case shown to depend upon sub-units, then the term plasmagene should be shifted from the structure as a whole to its gene-like component parts. Meanwhile I shall employ the term plasmagene for those cytoplasmic structures known to manifest genic properties, however infelicitous this may seem when applied to bodies large enough to be microscopically visible.

(c) *Relation to the nuclear genes*

The proof that a difference in hereditary traits is due to a difference in cytoplasm necessarily involves the demonstration that the organisms manifesting the diverse traits have identical genomes. If they had diverse genomes, the observed difference in traits obviously might be due to the gene differences. From the identity in genomes the conclusion is often drawn that the cytoplasmic basis of heredity, the plasmagene, is independent of the genes. Yet this conclusion is neither logical nor defensible. The genes may constitute a necessary, but not a sufficient, factor in the formation, maintenance or reproduction of the plasmagenes.

How then can it be determined whether the plasmagenes are in any way dependent on the genes? Unfortunately, there is no invariably dependable method of obtaining an answer to this question. The only method that can be used is the method of gene substitution. With luck, this can prove that genes are involved, but it can never prove that genes are not involved. Let us make the point concrete with a few examples.

The plastids, which are the basis for at least some examples of cytoplasmically inherited variegation in plants, were long believed to be independent of the genes because diverse plastid types persist and multiply in association with the same genome. Renner's observations on *Oenothera* showed this view to be false (see Rhoades, 1946). He obtained hybrid plants carrying two kinds of plastids of diverse origin. In these hybrids both plastid types persisted and multiplied; but one kind did not develop or function normally. When the hybrids were selfed or crossed to certain other plants, the abnormal type of plastid recovered and functioned normally in some of the resulting genomes.

Thus, certain plastid types are adapted to definite intracellular conditions which the genes control. The illusion of gene-independence is dispelled when appropriate materials are investigated.

Interpretation of the relation of kappa to the genes in *Paramecium* passed through a similar history. The sensitives employed early in the work carried the *K* gene, as did of course the killers. Crosses between such materials showed no difference between killers and sensitives in any genes affecting the killer trait. Likewise, the irreversible destruction of kappa, transforming killers into hereditary sensitives, and the introduction of kappa into sensitives, transforming them into hereditary killers, indicated that kappa was independent of the genes. Only after four years did the lucky chance of finding strains carrying *k* reveal the hidden gene dependence: kappa disappears in the absence of gene *K*.

In view of this experience and of Renner's work on plastids, it should be clear that the failure to find a gene difference affecting a plasmagene is no guarantee that the plasmagene is gene independent (Sonneborn, 1943). Moreover, *a priori* considerations render improbable the possibility that such plasmagenes could persist and multiply except in cytoplasm of a definite constitution which could scarcely be free from decisive gene modification. I therefore doubt whether totally gene-independent cytoplasmic inheritance exists in any form, based on plasmagenes or otherwise.

However, the nature of the dependence of a plasmagene on the nuclear genes may not be as obvious as it seems. In the examples already discussed, the genes seem clearly to control the functioning or the maintenance, but not the initial production, of the plasmagene. The plasmagene disappears or manifests disfunction in cells of the "wrong" genotype, persists and functions normally in cells of the "right" genotype; but, once lost from the "right" genotype, it is not again produced. Cells with only defective plastids may have the same genes as cells with normal plastids, yet they do not produce normal plastids. *Drosophilæ* lacking sigma do not produce sigma, even when they have the same genes as sigma-bearing strains. Sensitive paramecia that lack kappa do not give rise to kappa, although they may have gene *K* and every other gene present in killer paramecia. The essential genes in these cases seem not to function in the initiation of the plasmagenes. On the other hand, the problem of whether the genes can initiate formation of a plasmagene is, in a sense, like the problem of discovering whether plasmagenes are ever gene-independent. Failure to demonstrate it may be due to methodological failure. As Waddington (1948) has pointed out, the very fact that a cell has developed in one way may make it practically impossible to detect whether it could have developed in another way. The internal conditions may no longer be comparable to the ones that prevailed when the alternative pathway was still a practicable possibility. While such considerations make judgments uncertain,

nevertheless, in the absence of evidence to the contrary, it seems unlikely that the genes can initiate production of plastids, kappa, sigma or the like.

The dependence of plasmagenes on nuclear genes raises two further questions about them : one concerning their relative importance and the other, their evolution. It has been maintained, on various grounds, that plasmagenes occupy in the cell hierarchy a rank lower than the genes (Beadle, 1949). Yet, so far as maintenance and normal functioning are concerned, the dependence of plasmagenes on genes is not obviously either greater than, or different from, the dependence of genes upon cytoplasm. Baltzer (1910) showed that certain chromosomes of the sea urchin, *Sphærechinus*, could not be maintained in the cytoplasm of *Strongylocentrotus* ; and he and others subsequently reported a number of comparable cases in other organisms. Similarly, certain chromosomes are regularly eliminated from definite cells of *Ascaris* (Boveri, 1899) and *Sciara* (Metz, 1931) ; and one chromosome of a variety of *Vicia faba* is unable to survive in homozygous form in the cytoplasm of another variety (Sirks, 1931). The cytoplasmic conditions involved here may be, and probably are, more or less affected by genes. There is no evidence of a reverse effect of cytoplasm on the character of the chromosomes. In this sense, the cytoplasm may be subordinate to the genes. Nevertheless, it is still clear that chromosome maintenance depends upon specific cytoplasmic constitution just as the maintenance of plasmagenes depends upon the proper chromosome constitution. Genes and cytoplasm are mutually and specifically dependent upon one another for their maintenance.

The evolution of plasmagenes has been reflected upon by numerous authors for more than twenty years. A recurrent suggestion has been that plasmagenes and nuclear genes may undergo parallel evolution. Renner's report that genetically different plastids occur in different *Oenotheras* and that the different plastid types are adapted to different genomes is in agreement with the notion of parallel evolution. I have preliminary indications of the same sort of thing in *Paramecium*. Killers occur in two varieties of *P. aurelia*, varieties 2 and 4, which cannot interbreed at all. A killer race of variety 2 was freed of kappa and thereby converted into a sensitive. It was then exposed to the macerated bodies of a variety 4 killer. This method, it will be recalled, is successful in introducing kappa into *KK* sensitives of variety 4, thereby transforming them into hereditary killers. But the attempts thus far made to use this method for introducing kappa from variety 4 into variety 2 have failed. Although the matter requires much further investigation, it is possible that the kappas in these two varieties, like the chloroplasts in Renner's *Oenotheras*, depend on different genomes for their normal maintenance. The genome of the variety 2 race can maintain its own kappa, but it may be unable to maintain the kappa from variety 4. It would be easy to account for such a condition by assuming first a mutation of kappa and then a gene

mutation which could support only the mutant, not the original, kappa. Some such course of events might also underly other examples, previously mentioned, of incompatibilities between chromosomes and cytoplasm. In other words, selected and correlated mutations may occur in nucleus and cytoplasm in much the same way as must occur in hosts and parasites if their association is to be preserved.

(*d*) *Transmission and rate of reproduction*

Some plasmagenes are distributed to the daughter cells in an essentially random manner at cell division. Preer (1948*a*) has pointed out that randomly distributed genetic particles, multiplying at the same rate as the cells in which they lie, must eventually be lost from an increasing proportion of the cell progeny. How is it then that randomly distributed plasmagenes manage to persist? Kappa may be taken as an example of a plasmagene which is distributed practically at random during cell division. Preer (1948*a*) has shown in this case the existence of compensatory mechanisms. When the cellular concentration of kappa drops, its rate of reproduction rises; when the cellular concentration of kappa rises, its rate of reproduction falls. This serves to keep the concentration fairly uniform under most ordinary conditions and thus cancels the risks of loss by random distribution.

Some plasmagenes, on the other hand, are not randomly distributed at cell division. The cinetosomes or ciliary basal granules of *Paramecium* and other ciliates remain in fixed position. There they duplicate and, at fission, every animal gets its full allotment of cinetosomes. There is no danger of loss by this method of distribution.

Still other plasmagenes are as precisely distributed as are the chromosomes by mitosis. The centriole divides when the cell divides and each daughter cell gets one. The gullet of *Paramecium* buds off a new gullet which severs its connection with the parental gullet and migrates to the proper position in the daughter cell (Hertwig, 1889). In certain organisms, the chloroplasts are precisely and equally distributed at cell division. The mitochondria are in some cases equally distributed; they may even undergo precise reduction of number at the time of the meiotic mitoses (Wilson, 1916).

The various methods of distribution of plasmagenes are thus all well adapted to preserving them. Indeed, there seems to be no fundamental difference between plasmagenes and nuclei in respect to method of distribution at cell division. Most nuclei are of course distributed equally and precisely, like centrioles and certain other plasmagenes. When there are normally many nuclei in a cell, as in some ciliated protozoa, they are distributed in the same manner as kappa, *i.e.*, randomly.

As already implied, the reproduction of some plasmagenes is not necessarily synchronised with nuclear and cell division. Under some

conditions, chloroplasts may divide more slowly than the cells that contain them (Ternetz, 1912). This of course results in fewer chloroplasts per cell or in none at all. Similarly, kappa multiplies less rapidly than the paramecia under certain conditions of feeding (Preer, 1946) and of temperature (Sonneborn, 1946). Conversely, under other conditions of feeding and temperature, the rate of multiplication of kappa may be greater than that of the cell until the maximal concentration is attained.

These aberrancies in plasmagene reproduction are also paralleled by similar aberrancies in the reproduction of nuclei and chromosomes. As is well known, cells can divide without nuclear division, nuclei can divide without cell division, and chromosomes can duplicate without either nuclear or cell division. The phenomena do not seem to be different in principle as between plasmagenes and nuclear genes. Under normal conditions, in both cases, there is usually a neat correlation in replication rates to preserve the *status quo* ; under aberrant conditions, these correlations are disturbed and either the plasmagenes or the nuclear genes may be lost, often with fatal consequences in either case. There must be strong selection under normal conditions for correlated replication rates of all essential self-duplicating cell structures.

(e) *Functions*

Beadle (1949) and some workers on the plasmon (see review by Caspari, 1948) maintain that plasmagenes have relatively unimportant functions in comparison with the nuclear genes. According to a common form of this view (see Caspari), all essential activities of the cell are controlled by the genes, plasmagenes doing nothing but interfering with gene action. For example, Schlösser (1935), who subscribes to this view, showed that one of the plasmon effects in *Epilobium* is to alter the osmotic pressure of the cells and thereby to inhibit the action of certain genes. But the osmotic pressure is itself an hereditary trait, which in this case at least seems to be determined by the plasmon. Moreover, inhibitory or modifying actions are certainly not uniquely characteristic of plasmagenes, for genes also act as specific inhibitors or modifiers of the action of other genes. Finally, there can be little doubt of constructive, as opposed to interfering, action when respiratory enzymes are cytoplasmically inherited (Ephrussi *et al.*, 1949) or when the plasmagene is an essential and non-replaceable structure like a chloroplast, a centriole, a cinetosome or the like.

(f) *Origin*

A common criticism of plasmagenes is that they are not normal integral parts of the genetic equipment of the cell, but rather symbionts or parasites of extrinsic origin. Lindegren (in Sonneborn, 1946) and Altenburg (1946) have maintained this with respect to kappa ;

L'Héritier (1948) has considered the same possibility for sigma. There are certainly reasons for suspicion. Kappa is infectious when in concentrated suspension (Sonneborn, 1948*a*) and, unlike normal cytoplasmic constituents as at present conceived, it contains desoxyribonucleic acid (Preer, 1948*b*, and in press); moreover, most paramecia contain no particles comparable to kappa. Sigma too is infectious by the techniques of transplantation and injection (L'Héritier, 1948). Both Sonneborn (1948*a*) and L'Héritier (1948) have emphasised, however, that these particles are infectious only under conditions that could scarcely occur in nature. Because of this, Darlington (1949*b*) refers to them as "proviruses." In my opinion (Sonneborn, 1948*a*, 1949), the question is largely verbalistic and tends to become academic. I believe Darlington, L'Héritier, and I are agreed that kappa and sigma are borderline cases and that, regardless of their origin, they are of genetic significance because they are normally transmitted only by heredity.

One cannot but be impressed by the fact that practically every self-duplicating structure occurring within cells has at one time or another been considered a symbiont or parasite. For example, chloroplasts and mitochondria have been interpreted as symbionts (see review by Buchner, 1921). Even the nuclear genes have not been spared. Thus Wallin (1927) claims to have demonstrated that mitochondria are symbiotic bacteria and he believes that they provide the chromosomes with the new genes necessary for evolution. However interesting they may be, all such speculations as to the possible external origin of self-duplicating intracellular bodies are, from the point of view of the present genetics of the organism, outweighed by the fact that at least some of them, such as plastids and other cell structures, are now normal parts of the genetic equipment of the cell.

(*g*) *Occurrence*

It has often been urged that very few plasmagenes with detected effects on hereditary traits are known, particularly in animals. By comparison with the number of known nuclear genes, this is certainly true. Yet it must be remembered that the detection of plasmagenic effects may be beset with special difficulties. Unlike lethal nuclear gene mutations, lethal plasmagene mutations could not be recognised at all. The phenomena of diploidy and meiosis, which preserve and reveal nuclear gene mutations, even lethals, have no known counterparts that would render possible the detection of lethal plasmagene mutations. If plasmagenes control very fundamental processes that cannot be altered without disastrous consequences, they would seldom be detected. We may not often expect so fortunate an arrangement as the dual system of respiration in yeast, without which it would not have been possible to discover the cytoplasmic inheritance of respiratory enzymes (Ephrussi *et al.*, 1949). Nevertheless, it is obvious

—even without employing ordinary genetic methods—that visible plasmagenes form much of the essential equipment of the cell. These are indeed widespread, common, even ubiquitous.

III

The specificity of the physical basis of cytoplasmic inheritance is in some cases controlled, not by the plasmagenes or the cytoplasm, but by the nuclear genes. This is commonly believed to be the case for the diverse cell types that arise during the development of higher animals from the fertilised egg, and that persist during repeated subcultures *in vitro* or in grafts. However, there is little critical evidence upon which to base a well-founded genetic interpretation of persistent developmental differentiations and, as a result, a number of fundamentally different interpretations have been suggested or repeated in recent years (Darlington, 1944 ; Sonneborn, 1943, 1947*b*, 1948*b*, 1949 ; Spiegelman, 1946 ; Waddington, 1948 ; Wright, 1941, 1945). The obvious limitations of genetic analysis of somatic cell differences stress the need for a comparable situation in material better suited to genetic analysis. This seems to be provided by our study—still in progress—of the determination and inheritance of antigenic types, or serotypes (Sonneborn, 1947*b*, 1948*b* ; Sonneborn and Le Suer, 1948 ; Beale, 1948 ; Sonneborn and Beale, 1949 ; and Sonneborn *et al.*, unpub.).

(*a*) *The main facts on determination and inheritance of serotypes in* P. aurelia, *variety* 4

After paramecia of a certain strain are injected into a rabbit, the rabbit's serum, even in high dilution, immobilises or paralyses paramecia of the same strain. This is a specific antibody-antigen reaction, for dilute antisera obtained from different rabbits injected with different strains of paramecia may be capable of immobilising only the homologous strain, *i.e.*, the one injected into the rabbit from which the serum was obtained. Each serologically distinct strain of paramecia is known as an antigenic type or serotype.

Each stock (consisting of the vegetative and sexual progeny derived exclusively from one homozygous individual) gives rise eventually to a series of diverse serotypes. The serotypes thus far found in stock 51 have been designated A, B, C, D, E, G, H and J ; those in stock 29 are A, B, C, D, F, H and J. F has not yet arisen in stock 51 and neither E nor G has arisen in stock 29. Moreover, serotypes A, C, D and H are not identical in the two stocks : antiserum against any one of these types, from either stock, will immobilise animals of the corresponding type from the other stock, but minor differences between the corresponding types are clearly demonstrable by serologic methods. Further study may reveal that the other types which occur in both stocks are also distinguishable, and it is practically

certain that more types will be found. Differences in the spectrum of possible serotypes, comparable to those that distinguish stock 29 from stock 51, are also found among the other stocks of variety 4 obtained from different sources in nature. The fact that every animal in each stock belongs to some serotype, indicates that these traits are normal and indispensable. As similar serotypes occur in stocks collected from Asia and America they are apparently ancient characters of the species.

The immobilisation of each serotype by its homologous antiserum seems to involve a single distinctive major antigen or antigen complex, which may be designated as its " specific immobilisation substance " (*SIS*). For example, serotype A is apparently not immobilised by the antibodies that immobilise any other serotype. Hence, the *SIS* of serotype A is not present, or at least it seems not to function in immobilisation, in any other serotype. Although the stock as a whole is capable of producing a whole spectrum of *SIS*, the presence of any one of them in any individual excludes more or less completely the presence of any other one. In a few serotypes, however, there is present not only the *SIS* of this serotype but also the substance which acts as a *SIS* in another serotype. The latter seems to be present in large amount, but it does not function in immobilisation. As will appear, mutual exclusion of specific immobilisation substances is of decisive importance in the theoretical interpretations.

Exposure of any serotype to its immobilising antiserum transforms it into other serotypes. Antiserum, along with temperature and other conditions, can direct the transformations to a particular type. For example, serotype D of stock 29 can be transformed by anti-D serum to type B at 32°, to type H at 20°, in each case about 95 per cent. of the treated individuals transforming. Some, indeed probably all, of these transformations are reversible. In stock 51, A and B are repeatedly convertible one into the other, and all other serotypes can easily be transformed to A or to B, as desired. In stock 29 serotypes B, F and H are readily interconvertible and any other serotype can be transformed to any of these. Most of the remaining transformations are not yet under experimental control, but they may yet be achieved. It seems therefore that irreversible losses of potencies do not take place in the process of transformation.

The serotypes are inherited under standard conditions of culture (26° to 27° C. with enough food to permit one fission per day). Spontaneous changes of serotype rarely occur under these conditions. When spontaneous or induced changes do occur, the new types are inherited thereafter under the standard conditions of culture. Crosses made among different serotypes of the same stock reveal no effective gene differences among them : all the serotypes of one stock are identical in genes. As this suggests and as further analysis shows, the serotype differences follow the cytoplasm in inheritance. Hereditary transformations of serotype are thus cytoplasmic " mutations " of hitherto unknown type.

But the genes also play a part in the control of serotypes as appears when different *stocks* are crossed. It will be recalled that the specific immobilisation substance of serotype A is not quite identical in stocks 29 and 51. Crosses between the two stocks show that the specific difference between their A substances is controlled by a pair of allelic nuclear genes with the 51A gene dominant over the 29A gene. This is the basis for concluding that nuclear genes control the specificity of the physical basis of cytoplasmic inheritance in these antigenic traits.

Single gene differences have also been found to control other differences between the stocks. For example, serotype F is one to which any serotype of stock 29 can be readily transformed ; but we have not succeeded in obtaining type F under any conditions in stock 51. The capacity to transform to type F under the conditions employed depends upon a gene present in stock 29 and absent from stock 51. Serotype H is another to which any serotype of stock 29 can be easily transformed under defined conditions ; but methods for achieving this in stock 51 are unknown. The capacity to transform to type H under the conditions employed depends upon another gene present in stock 29 and absent from stock 51.

The results with serotype H are instructive. They were obviously parallel to the results with type F and we at first believed they meant that genes for the F and H specific substances were lacking in stock 51. It was not until much later that type H was found for the first time in stock 51. Obviously the gene we were following has nothing to do with the capacity to form the H substance ; it merely controls the response to particular transforming conditions. For example, at low temperatures, stock 29 serotypes transform to types F and H while stock 51 serotypes transform mainly to type B. In view of these new findings the F gene previously followed is probably of the same sort and tells nothing about the capacity of stock 51 to yield type F.

Indeed other evidences from serologic and genetic experiments indicate that stock 29 also has the genes required for control of the specific E and G substances, although these serotypes have never been found in stock 29. I therefore believe that stocks 51 and 29 both have the genotype required for production of all the serotypes A to J. On this view the differences between the stocks are of two kinds : first, corresponding serotypes are slightly different as a result of different specificity alleles ; second, corresponding serotypes are called forth or stabilised under different conditions, the response system being controlled also by genes.

To summarise, the main facts brought out by this survey are : (1) Each homozygous stock is characterised by a spectrum of possible serotypes. (2) Different stocks are characterised by different spectra of possible serotypes. (3) The specific immobilisation substance of each serotype more or less completely excludes the presence or functioning (in the immobilisation reaction) of any other specific immobilisation substance. (4) Environmental conditions (including

homologous antiserum) bring about directed transformations of serotype. (5) Many, possibly all, of the transformations are reversible, indicating that no potencies are lost in the process of transformation. (6) The differences among the serotypes in any one stock are due not to genic, but to cytoplasmic, differences which are inherited under the standard cultural conditions. (7) The specific differences between corresponding immobilisation substances in different stocks are determined by allelic genes. (8) Each stock of variety 4 probably has a full set of specificity genes for all the serotypes found in the stocks of this variety. (9) The stocks differ in other genes controlling the response to particular transforming conditions (*i.e.*, which serotype will arise).

(*b*) *Comparison of the serotype and killer systems of inheritance*

The system of determination and inheritance of the serotypes probably reflects a fundamentally different physical basis and mechanism of cytoplasmic inheritance, because it differs so strikingly from the system exemplified by the killer trait and set forth in part II of this paper. To be sure, the two systems are alike in some features : in both, traits are cytoplasmically determined and inherited ; in both, environmental conditions can bring about hereditary changes ; in both, the cytoplasmically inherited traits depend for their maintenance on nuclear genes. But there the resemblances end.

The differences appear to be of four main kinds. First, the specific type of killer trait is controlled by the kind of kappa present, not by the genes, as Dippell's analysis of kappa mutations demonstrated. On the other hand, the specific type of A immobilisation substance is determined by the nuclear genes. Second, transformations from killer to sensitive are readily made irreversible, while transformations of serotype are not. Third, the various types of killer character are not mutually exclusive, as Dippell's study of the mutants shows : different kinds of kappa can coexist in the same animal and its progeny, each kind of kappa controlling production of its corresponding kind of paramecin. In the serotypes, on the other hand, two kinds of specific substances, both functioning as immobilisation substances, cannot coexist and maintain themselves in a line of descent. They are mutually exclusive. Fourth, the physical basis of the killer trait lies in the visible, Feulgen-positive kappa particles. Search for such particles associated with the serotypes has failed to reveal them. These fundamental differences between the killer and serotype systems must be taken into account in attempts at interpretation.

(*c*) *Interpretations of the serotype system*

(1) *The hypothesis of gene-initiated plasmagenes.*—Cytoplasmic inheritance is naturally explained by plasmagenes, just as nuclear inheritance is by chromosomal genes. The fact of genic control of specificity in

the serotype system restricts plasmagenic interpretations to forms different from the one discussed in the first part of this paper. In this section, we consider the hypothesis that the specificity-controlling genes produce the specific immobilisation substances which are the plasmagenes. This was adopted by Sonneborn (1948*b*) and Beale (1948) as a working hypothesis and it had the merit of leading to the discovery of a number of the facts. It is based on an idea which has been considered by Wright (1941), Darlington (1939, 1944), and Spiegelman (1946), and which is at least as old as De Vries' pangenes.

On this hypothesis, the genic origin of the specific substances is suggested by the genic control of specificity. The reversibility of transformations is due to the permanence of the genic source of the plasmagenes. The efficacy of specific antiserum in bringing about transformations is due to interference with plasmagene reproduction as a result of the combination of plasmagene with specific antibody. To account for mutual exclusion and the environmental control of the direction of transformation, an additional assumption is required. As this requirement is common to several of the hypotheses, it will be discussed later.

The hypothesis of gene-initiated plasmagenes meets a serious difficulty in accounting for the results of the cross between 51A and 29A. It will be recalled that the 51A gene behaved as a dominant. This means that all of the F_1 were of serotype 51A, even those (derived from one member of each pair of mates) whose cytoplasm was carrying the full measure of 29A substance before fertilisation. This seems to be inconsistent with the plasmagene hypothesis. If the specific 29A substance is a plasmagene, it is by definition capable of multiplication and should not disappear. To be sure, in the hybrid the 51A substance should also arise under the action of the 51A gene. A mixture of 51A and 29A substances is therefore expected in the vegetative progeny. But the 29A substance is found only in one member of each conjugant pair and only for a few fissions, after which it can no longer be detected. Even if dominance in this case is understood as suppression of the action of the 29A gene by its 51A allele, the results are not explained. This would merely cut off the further production of 29A plasmagenes by the gene ; it would not account for the apparent failure of multiplication by the plasmagenes already present.

In view of the F_1 results, the observations in the F_2 are not surprising. Each clone develops the serotype corresponding to its genes. Those that carry the 51A gene remain 51A ; those that become homozygous for the 29A gene lose the 51A substance in a few fissions and transform to 29A. This shows that the results in the F_1 could not be due to suppression of the 29A plasmagene by the 51A plasmagene, for the reverse occurs in the F_2. Moreover, there is no obvious difference in the rate at which the two opposite changes occur.

Thus the 29A substance quickly disappears in the presence of the 51A gene, even when the 29A gene is also present; and the 51A substance disappears just as quickly in the presence of the 29A gene, when the 51A gene is absent. This can hardly be interpreted otherwise than as a conspicuous failure of the gene-initiated plasmagene hypothesis. And a more favourable opportunity for such plasmagenes to demonstrate their existence could scarcely be conceived.

Negative evidence is of course never quite convincing. Yet this is the second failure of the hypothesis. A few years ago, Spiegelman, Lindegren and Lindegren (1945) claimed that the adaptive enzyme, melibiozymase, in yeast was maintained and multiplied in the presence of substrate and in the absence of the gene required for its initial formation. If this claim had been confirmed, it would have gone far toward establishing the existence of gene-initiated plasmagenes. Naturally the work attracted an enormous amount of interest and was the basis for widespread acceptance of the concept. However, attempts to repeat the observations have failed (Lindegren and Lindegren, 1946).

Our own failure is even more striking because the assumed plasmagene was lost in the F_2, in the presence of a gene with an effect almost identical with that of the controlling gene; and because it was lost in the F_1 *even in the presence of the controlling gene itself*. Thus, if gene-initiated plasmagenes are involved here, they must also depend on the same gene, subject to the rules of dominance, for their maintenance. Why this should be is not at all evident. It would seem more likely that *other* genes would control the cell conditions affecting survival or multiplication of the plasmagene. If the same gene that is assumed to be producing the plasmagene must *continually* act to assure its survival and multiplication, it might as well be assumed that the specific substance is formed under gene action without the intervention of plasmagenes at all. With respect to the serotypes, therefore, the existence of gene-initiated plasmagenes is no longer even a useful assumption.

The other evidence cited for gene-initiated plasmagenes is the apparent autocatalytic increase of the substances involved. But autocatalytic increase is well known in systems devoid of a genetic component, so evidence of this sort cannot alone be considered decisive. We are thus driven to conclude that critical evidence for gene-initiated plasmagenes is entirely lacking.

(2) *The hypothesis of precursor as plasmagene.*—Wright (1945) suggested that genes may act by conferring specificities on plasmagenes. The specificity genes in serotype determination could act in that way, converting an indifferent precursor, with plasmagenic properties, into a specific immobilisation substance, but one that *lacks* plasmagenic properties. With this hypothesis the facts that rule out gene-initiated plasmagenes are consistent. The detected trait, *i.e.*, the specific immobilisation substances, would depend directly on the genes present,

just as they do. Unfortunately, however, the hypothesis effectively puts the plasmagene beyond the reach of present evidence, because the precursor is not directly detectible by any methods yet used.

Nevertheless, something can be said about the precursor, particularly as to whether there is one common to all the immobilisation substances or whether there is a unique precursor for each specific substance and its analogues in different stocks. Since the purpose of postulating a plasmagene is to account for cytoplasmic inheritance, there must be a different plasmagene for each of the alternative cytoplasmically inherited traits. Hence, in each stock one must assume a different precursor for each of its possible specific immobilisation substances.

This leads to predictions that might eventually be tested. First, it should be possible to remove one serotype from the spectrum of possibilities of a stock by destroying one of the precursors, as kappa was destroyed. Second, if the serologic properties of the specific immobilisation substances are determined in part by the nature of the precursor, then mutations of the plasmagene should be detectible. Third, in the same way corresponding precursors in different stocks must sometimes differ and the sort of observations made in the cross of 51A by 29A should show whether precursor multiplies in the absence of its original specificity gene. The two latter predictions could be tested only by finer serologic distinctions than any yet recognised in our work.

(3) *The hypothesis of variable gene activity.*—Kimball (1947) suggested that the rate at which a gene functions might vary directly with the cellular concentration of its direct or indirect product. This would result in cytoplasmic inheritance, for high concentration of such a product would call forth from the genes the action required to maintain it in high concentration. On this view the specific immobilisation substances themselves, or specific precursors of each, are the stimuli for accelerating the activity of the controlling genes. The transforming action of antiserum would then be interpreted as due to the failure of specific-substance-antibody complex to stimulate the gene in the way done by the specific substance alone.

(4) *The need for an additional assumption.*—The three hypotheses we have considered all fail, without an additional assumption, to account for two of the most striking facts in the serotype system. One of these is the mutual exclusion phenomenon, *i.e.*, the fact that in each of the serotypes only one of the several potentially producible substances is present as a specific immobilisation substance. The other is the environmental control of the direction of transformation, *i.e.*, the fact that a particular specific immobilisation substance arises under given transforming conditions. To account for these two facts, either one of two additional assumptions may be made.

First, competitive processes may be assumed (Sonneborn, 1948*b*). Depending on the hypothesis, the competition would be among the

plasmagenes or among the gene products that stimulate gene activity. The competition could be for sources of energy, for simpler substances used in synthesis of the specific substances, or for position in the cell. Control of the direction of transformation by environmental conditions would then be an expression of environmental influence on the competitive process.

Second, the presence of any one of the specific substances (or of a precursor of it) in high concentration may inhibit some step in the synthesis of all the other specific substances (Delbrück, in Sonneborn and Beale, 1949).

Not only does such a system of mutual inhibition account for mutual exclusion and directed environmental transformations, but, as Delbrück clearly showed, it also accounts for cytoplasmic inheritance without resort to plasmagenes. For, so long as the inhibitor is present in high concentration, it is perpetuated by excluding the alternative substances.

Doubtless many variations on this theme could be imagined. For example, Waddington (1948) has indicated how competition for substrates, combined with autocatalysis of the products, could yield a system capable of developing along any one of several mutually exclusive lines and, at the same time, automatically perpetuating the alternative actually realised.

(5) *Conclusions as to interpretations.*—Of the several hypotheses considered as explanations of the serotype system, the hypothesis of gene-initiated plasmagenes has been strongly discredited ; but the others are all more or less satisfactory. Two considerations favour the hypothesis of gene-specified plasmagenes (supplemented with a competition or inhibition assumption). First, some of the discoveries made in the serotype work were predicted on the basis of plasmagenes and competition ; these assumptions have therefore justified themselves as a working hypothesis. Second, plasmagenes are known to be the basis of cytoplasmic inheritance in a number of cases, as pointed out in the first part of this paper, while not a single example of cytoplasmic inheritance has yet been shown to be based on variable gene activity or mutual inhibitions or competition and autocatalysis. On the other hand, either of the two latter alternatives may be preferred because they are simpler, dispensing both with plasmagenes and variable gene activity and retaining only an accessory assumption required by the other, more complex hypotheses. However, there is no critical evidence that would exclude any one of these hypotheses. If any one of the three predictions set forth in the discussion of the precursor-plasmagene hypothesis were verified, this would constitute such critical evidence.

This will, I am afraid, seem like a most unsettled and unsatisfactory state in which to leave the remarkable system of serotype determination and inheritance, particularly after such a lengthy theoretical discussion. But we are studying a type of cytoplasmic inheritance never before

subjected to this sort of intensive experimental analysis. And the investigation is still young ; theoretically decisive results may yet be expected.

The main justification of the discussion, however, is its possible relation to normal developmental differentiation. The spectrum of serotypic possibilities inherent in each stock of paramecia may bear more than a superficial resemblance to the spectrum of cellular possibilities inherent in the fertilised egg. To the nine facts of the serotype system listed on pp. 25 and 26, there are either known or probable parallels in normal developmental differentiation, if the inductors and evocators of development are substituted for the environmental agents used to transform *Paramecium*. Only concerning the fact of reversibility can there be serious doubt and recent work of Rose (1948) and others shows that even this doubt should not be too strong.

IV. SUMMARY

Cytoplasmic inheritance is one of the capital facts of biology. It occurs in at least two forms that seem to be fundamentally different. One of them is based on self-duplicating, mutable, cytoplasmic particles—the plasmagenes—which depend on the nuclear genes for their maintenance or normal functioning, but not for their origin or for their specificity. These plasmagenes, in different cases, vary in size from microscopically visible particles down to submicroscopic particles probably of the same order of size as the genes. There are indications that at least some of the larger plasmagenes contain or consist of smaller genetic units. Plasmagenes are probably never independent of the genes, but this does not support the conclusion that they occupy in the cell hierarchy a position inferior to the genes. Genes and cytoplasm are mutually interdependent for their maintenance and normal functioning. Evolution may proceed by parallel but independent mutations of nuclear genes and plasmagenes. Plasmagenes have varied methods of transmission and varied rates of duplication, but in all cases the system is well adapted to their preservation. Far from merely interfering with normal gene action, plasmagenes control vital and constructive activities. While the origin of plasmagenes is obscure and may in some cases be external, some are at present normal and important parts of the cell organisation. Many examples of this sort of plasmagene are known and they may occur even more widely than is yet evident.

About the other form of cytoplasmic inheritance, manifested by serotypes of *Paramecium* and the somatic cell differences in higher animals, much less is known. This form is distinguished from the first one in several ways, chief among which is the genic control of the specific nature of the character that is cytoplasmically transmitted. Although a well-defined system of facts is known for this type of cytoplasmic inheritance, its physical basis and mechanism remain to

be discovered. Of the various possibilities, that of plasmagenes produced by genes seems to be excluded, but another type of plasmagene hypothesis and other hypotheses in which plasmagenes play no part remain to be tested.

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FIG. 1. FIG. 2.

FIG. 1.—A sensitive *Paramecium*, Giemsa stain, showing macronucleus and a food vacuole containing bacteria, but no kappa particles in the cytoplasm. × 1430.

FIG. 2.—A killer *Paramecium*, Giemsa stain, showing the macronucleus and the large number of kappa particles in the cytoplasm. × 1430.

METHODS OF SCORING LINKAGE DATA GIVING THE SIMULTANEOUS SEGREGATION OF THREE FACTORS

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1. INTRODUCTION

WHEN estimating the values of a number of recombination fractions for various segments of a chromosome, from linkage data, it is often the case that more than one body of data is available. The data may relate to various sources, different types of crosses and consist of parts supplying information for one or more of the segments. In such cases, it is, obviously, desirable to combine the data in order to make joint estimation of the parameters. The estimates, thus obtained, are the most efficient when different parts of the data are homogeneous which can, however, be directly verified by testing whether the estimates closely fit in with the various parts of the data.

Fisher (1935) put forward a method of scoring which seems fitted for general use where combination of data, tests of homogeneity and pooled estimates are considered. This method consists of replacing each body of data by appropriate scores and information which are directly used in arriving at pooled estimates and tests of homogeneity. In a paper Fisher (1946) used this method of scoring in estimating three recombination fractions (arising out of two consecutive segments and subject to Kosambi's (1944) restriction) from data which in parts supply information only for the individual segments. If the data giving the simultaneous segregation of three or more factors are available then two questions arise. What is the most appropriate method of scoring such data and how does it compare with the alternative method of scoring for each recombination fraction by considering the classification with respect to the two relevant factors only and ignoring the rest? The latter method of scoring may be called the *individual segment method.*

When the simultaneous segregation of more than two factors has been recorded, the scores obtained by the individual segment method are less efficient in the sense in which Fisher (1935) defines efficient scores. The estimates to which they lead differ from the maximum likelihood estimates and hence are less efficient than the best estimates. The purpose of this article is to study these problems in the simplest case of scoring data on three factors arranged in eight phenotypical classes.

Firstly, a method appropriate and efficient for such data has been developed for the estimation of recombination fractions in the two different situations, viz. (1) when they are taken as free parameters and (2) when they conform to Kosambi's formula. Secondly, the relative efficiencies of the estimates in each of the two cases obtained by the individual segment method as compared with the above method have been calculated in a particular case to measure the loss in efficiency due to the individual segment method. The method of efficient scores involves a slightly heavier computational procedure and, though undoubtedly more efficient, can be recommended in practice only when even a small gain in efficiency is of considerable importance.

2. THEORETICAL ASPECTS OF THE METHOD OF SCORING AND NOTATIONS

Let there be k sets of data, each part supplying information on one or more of a set of p unknown parameters $\theta_1, \theta_2, \ldots, \theta_p$. If $L_1, L_2, \ldots L_k$ represent the probability densities of the observations corresponding to the k sets of data then L, the likelihood of the parameters, is defined by the product,

$$L = L_1 L_2 \ldots L_k$$

The best estimates of the parameters are those values of $\theta_1, \ldots, \theta_p$ which maximise the above expression. A formal proof of this statement is given elsewhere (Rao, 1947). For convenience the logarithm of L may be maximised. Differentiating log L partially with respect to $\theta_1, \ldots, \theta_p$ and equating to zero we get

$$\frac{\partial \log L}{\partial \theta_i} = \frac{\partial \log L_1}{\partial \theta_i} + \ldots + \frac{\partial \log L_k}{\partial \theta_i} = 0$$

$$i = 1, 2, \ldots, p$$

as the equations giving the desired estimates.

The above equations are usually non-linear and hence the direct evaluation of the estimates is difficult. A general method is to start with trial solutions and derive linear equations giving small corrections to the trial values. The process may have to be repeated until the corrections become negligible. If $d\theta_1, \ldots, d\theta_p$ are additive correc-

tions to trial values $\theta_1^0, \ldots, \theta_p^0$ one derives the equations giving the corrections as

$$\frac{-\partial^2 \log L}{\partial\theta_i \partial\theta_1} d\theta_1 - \frac{\partial^2 \log L}{\partial\theta_i \partial\theta_2} d\theta_2 - \ldots = \frac{\partial \log L}{\partial\theta_i}$$

$$i = 1, 2, \ldots, p$$

where all the derivatives are calculated at the trial values. If the sample is large we may replace $-\partial^2 \log L/\partial\theta_i\partial\theta_j$ by its mean value which is the same as I_{ij} the mean value of $\left(\frac{\partial \log L}{\partial\theta_i} \cdot \frac{\partial \log L}{\partial\theta_j}\right)$ at the trial values. The matrix $I = (I_{ij})$ is called the information matrix for the whole body of data at the assumed values.

The quantity $\partial \log L/\partial\theta_i$ is defined as the i-th efficient score and since

$$\frac{\partial \log L}{\partial\theta_i} = \frac{\partial \log L_1}{\partial\theta_i} + \ldots + \frac{\partial \log L_k}{\partial\theta_i}$$

it follows that the efficient scores for the whole data are the sums of the corresponding scores for the several sets of data. Similarly

$$I_{ij} = I_{ij}^{(1)} + \ldots + I_{ij}^{(k)}$$

where $I_{ij}^{(r)}$ is an element of the information matrix for the r-th part of the data.

Thus the problem of estimation reduces to replacing each part of the data by the efficient scores and information matrix at some trial values and finally obtaining the corresponding quantities for the whole data by simple addition. These supply linear equations in small additive corrections to the trial values.

It has been demonstrated in section 6 that the method of scoring offers a quickly converging process and hence is extremely useful in practice.

The scores for various parts of the data calculated at the best estimates are directly useful in tests of homogeneity as explained in sections 4 and 5 and illustrated in section 6. The theoretical aspects of such tests are fully discussed by the author in (Rao, 1948).

Notations.—

y_1, y_2, y_3 represent the recombination fractions in the segments connecting the second and third, third and first and first and second loci respectively.

$\dot{y}_1, \dot{y}_2, \dot{y}_3$ are the maximum likelihood estimates when they are treated as free parameters.

$\ddot{y}_1, \ddot{y}_2, \ddot{y}_3$ are the corresponding estimates when Kosambi's formula connecting y_1, y_2, y_3 is used.

$\phi_1^{(k)}$, $\phi_2^{(k)}$, $\phi_3^{(k)}$ are the efficient scores for γ_1, γ_2, γ_3 treated as free parameters from the k-th part of the data. When the data is arranged as frequencies in certain classes, it is known that

$$\phi_r^{(k)} = \mathrm{S}\left(\frac{n}{\pi}\frac{\partial \pi}{\partial \gamma_r}\right)$$

where S stands for summation, n for the frequency of a class and π for the corresponding probability. The total scores for the whole body of data are represented by Φ_1, Φ_2, Φ_3.

ψ_1, ψ_3 represent the scores for γ_1 and γ_3 when Kosambi's formula is used.

$(i_{rs}^{(k)})$ and $(\mathrm{I}_{rs}^{(k)})$ represent the information matrix per single observation and for the whole sample in the k-th part of the data. If n_k is the sample size then the relation $(\mathrm{I}_{rs}^{(k)}) = n_k(i_{rs}^{(k)})$ is identically true. It is known that,

$$i_{rs}^{(k)} = \mathrm{S}\left(\frac{1}{\pi}\frac{\partial \pi}{\partial \gamma_r}\right)\frac{\partial \pi}{\partial \gamma_s} = \mathrm{S}\frac{\partial \pi}{\partial \gamma_r}\left(\frac{1}{\pi}\frac{\partial \pi}{\partial \gamma_s}\right)$$

(T_{rs}) or simply denoted by $\mathbf{T}$ stands for the total information matrix $(\mathrm{T}_{rs}) = \mathrm{S}(\mathrm{I}_{rs}^{(k)})$.

The inverse of $\mathbf{T}$ is denoted by $\mathbf{T}^{-1}$. The method of finding this is to solve the equations

$$\begin{aligned} p\mathrm{T}_{11}+q\mathrm{T}_{12}+r\mathrm{T}_{13} &= 1, 0, 0 \\ p\mathrm{T}_{12}+q\mathrm{T}_{22}+r\mathrm{T}_{23} &= 0, 1, 0 \\ p\mathrm{T}_{13}+q\mathrm{T}_{23}+r\mathrm{T}_{33} &= 0, 0, 1 \end{aligned}$$

and take the three sets of equations as the three rows of $\mathbf{T}^{-1}$. $\mathbf{j}$ and $\mathbf{J}$ stand for information matrices per single observation and for the whole body of data when Kosambi's formula is used. All the above quantities calculated at $\dot{\gamma}_1$, $\dot{\gamma}_2$, $\dot{\gamma}_3$ and $\ddot{\gamma}_1$, $\ddot{\gamma}_2$, $\ddot{\gamma}_3$ are represented with a single dot and two dots above respectively.

3. SCORING OF DATA FROM BACK CROSSES AND INTERCROSSES

The eight different gametes due to a triply heterozygous parent can be classified into four complementary pairs, the members of each pair having equal chance of being transmitted to an offspring. Let the recombination fractions for the segments connecting the first and second, second and third and third and first loci be represented by γ_3, γ_1 and γ_2. Also let S_0, S_1, S_2 and S_3 represent respectively the frequencies of gametes involving all three recessive genes, only the first, only the second and only the third recessive gene. The S's and γ's are interrelated in the four types of heterozygotes as given in table 1.

TABLE 1

S functions for the four types of triple heterozygotes

Function of y's	Gametic frequencies for heterozygotes			
	$\frac{ABC}{abc}$	$\frac{AbC}{aBc}$	$\frac{ABc}{abC}$	$\frac{aBC}{Abc}$
$\frac{1}{4}(2-y_3-y_1-y_2)$	S_0	S_2	S_3	S_1
$\frac{1}{4}(y_2+y_1-y_3)$	S_3	S_1	S_0	S_2
$\frac{1}{4}(y_3+y_1-y_2)$	S_2	S_0	S_1	S_3
$\frac{1}{4}(y_3+y_2-y_1)$	S_1	S_3	S_2	S_0

For a given set of recombination fractions one can, by using the above table, calculate the S-functions for any type of heterozygote and use them for further calculations as the probabilities, scores and information matrix, in any particular situation directly depend on them.

(a) *Triple back cross*

The probabilities of the eight phenotypical classes and the appropriate scores, expressed in terms of S-functions, in the case of a back cross of a triple heterozygote with a triple recessive are given in table 2.

TABLE 2

Probabilities and scores in the case of a triple back cross

Phenotype	Probability	Scores for y_1	Scores for y_2	Scores for y_3	Observed frequency
ABC	S_0	$l_0/4S_0$	$m_0/4S_0$	$n_0/4S_0$	n_{111}
AbC	S_2	$l_2/4S_2$	$m_2/4S_2$	$n_2/4S_2$	n_{101}
ABc	S_3	$l_3/4S_3$	$m_3/4S_3$	$n_3/4S_3$	n_{110}
Abc	S_1	$l_1/4S_1$	$m_1/4S_1$	$n_1/4S_1$	n_{100}
aBC	S_1	$l_1/4S_1$	$m_1/4S_1$	$n_1/4S_1$	n_{011}
abC	S_3	$l_3/4S_3$	$m_3/4S_3$	$n_3/4S_3$	n_{001}
aBc	S_2	$l_2/4S_2$	$m_2/4S_2$	$n_2/4S_2$	n_{010}
abc	S_0	$l_0/4S_0$	$m_0/4S_0$	$n_0/4S_0$	n_{000}

The l's, m's and n's of the above table are four times the derivatives of probabilities with respect to y_1, y_2 and y_3 respectively and their values are determined by the following rule. The value of the derivative of the probability for a phenotype with respect to y_i is $-\frac{1}{4}$ or $\frac{1}{4}$ according as the letters other than the i-th in the representation of the phenotype is an old or a new combination, the old combinations being determined by the representation of the triple heterozygote. Thus in deciding the values of l_0, l_2, l_3 and l_1, one need only find which of the combinations BC, bC, Bc, bc, are old and which are new and take the l's corresponding to old combinations as -1 and the rest as $+1$. The values of these coefficients for the various types of heterozygotes are given in table 3 for ready use.

The efficient scores at any given values of y_1, y_2 and y_3 are obtained by summing the products of observed frequencies and scores. The

TABLE 3

Values of the l's, m's *and* n's *of table 2*

Heterozygote	Values of the coefficients
ABC/abc	$l_0 = l_1 = m_0 = m_2 = n_0 = n_3 = -1$
	$l_2 = l_3 = m_1 = m_3 = n_1 = n_2 = 1$
AbC/aBc	$l_2 = l_3 = m_0 = m_2 = n_1 = n_2 = -1$
	$l_0 = l_1 = m_1 = m_3 = n_0 = n_3 = 1$
Abc/aBC	$l_0 = l_1 = m_1 = m_3 = n_1 = n_2 = -1$
	$l_2 = l_3 = m_0 = m_2 = n_0 = n_3 = 1$
ABc/abC	$l_2 = l_3 = m_1 = m_3 = n_0 = n_3 = -1$
	$l_0 = l_1 = m_0 = m_2 = n_1 = n_2 = 1$

elements of the information matrix (i_{rs}) per single observation for given values of y_1, y_2, y_3 are the same for all types of back crosses. They can be simply calculated by using the formulæ

$$\begin{aligned} i_{rr} &= \tfrac{1}{2}(\lambda_0+\lambda_1+\lambda_2+\lambda_3) \quad r = 1, 2, 3 \\ i_{12} &= \tfrac{1}{2}(\lambda_0-\lambda_1-\lambda_2+\lambda_3) \\ i_{23} &= \tfrac{1}{2}(\lambda_0+\lambda_1-\lambda_2-\lambda_3) \\ i_{13} &= \tfrac{1}{2}(\lambda_0-\lambda_1+\lambda_2-\lambda_3) \end{aligned}$$

where $\lambda_0 = 1/(2-y_1-y_2-y_3)$, $\lambda_1 = 1/(y_2+y_3-y_1)$, $\lambda_2 = 1/(y_1+y_3-y_2)$ and $\lambda_3 = 1/(y_1+y_2-y_3)$. It may be noted that the λ's are fixed functions of the recombination fractions and any S-function assumes one of the values of λ for a given heterozygote. These have been introduced merely to simplify computations.

If the whole data consist only of results from back crosses the maximum likelihood estimates can be obtained without the evaluation of the efficient scores. It is easily seen that for such data the equations giving the best estimates are

$$\begin{aligned} (0)\lambda_0+(1)\lambda_1-(12)\lambda_2-(2)\lambda_3 &= 0 \\ (0)\lambda_0-(1)\lambda_1+(12)\lambda_2-(2)\lambda_3 &= 0 \\ (0)\lambda_0-(1)\lambda_1-(12)\lambda_2+(2)\lambda_3 &= 0 \end{aligned}$$

where (0), (1), (2) and (12) are the totals of observed frequencies from all sets of data corresponding to no cross overs, cross overs in the first segment only, cross overs in the second segment only and double cross overs respectively.

These equations yield the solutions

$$(0)\lambda_0 = (1)\lambda_1 = (12)\lambda_2 = (2)\lambda_3$$

or, writing in terms of the y's, they become

$$\frac{(0)}{2-y_1-y_2-y_3} = \frac{(1)}{y_2+y_3-y_1} = \frac{(12)}{y_1+y_3-y_2} = \frac{(2)}{y_1+y_2-y_3} =$$
$$\frac{(1)+(12)}{2y_3} = \frac{(2)+(12)}{2y_1} = \frac{(1)+(2)}{2y_2} = \frac{(0)+(1)+(2)+(12)}{2} = \frac{N}{2}.$$

Hence $\dot{y}_1 = \dfrac{(2)+(12)}{N}$, $\dot{y}_2 = \dfrac{(1)+(2)}{N}$, $\dot{y}_3 = \dfrac{(1)+(12)}{N}$.

The above estimates for y_1, y_2 and y_3 are the same as those obtained by considering the data as classified according two factors each time. Thus $\dot{y}_1$ could be obtained by considering only the second and third factors and ignoring the classification due to the first factor. Thus a complete classification with respect to three factors in the case of back crosses does not supply additional information so far as the problem of estimation of recombination fractions in various segments is concerned. This is true with more than three factors also.

The variances and covariances of the above estimates are

$$V(\dot{y}_1) = \frac{y_1(1-y_1)}{N}, \quad V(\dot{y}_2) = \frac{y_2(1-y_2)}{N}, \quad V(\dot{y}_3) = \frac{y_3(1-y_3)}{N}$$

$$\text{Cov. } (\dot{y}_1\dot{y}_2) = \frac{y_1+y_2-y_3-2y_1y_2}{2N}, \quad \text{Cov. } (\dot{y}_2\dot{y}_3) = \frac{y_2+y_3-y_1-2y_2y_3}{2N}$$

$$\text{Cov. } (\dot{y}_1\dot{y}_3) = \frac{y_1+y_3-y_2-2y_1y_3}{2N}$$

(*b*) *Intercross of a triple heterozygote*

The frequencies and their derivatives in terms of S-functions for any type of intercross are given in table 4.

TABLE 4

Frequencies and their derivatives for an intercross

Phenotype	Probability	Derivatives		
	π	$\dfrac{\partial\pi}{\partial y_1}$	$\dfrac{\partial\pi}{\partial y_2}$	$\dfrac{\partial\pi}{\partial y_3}$
ABC	$\frac{1}{4}+S_0+S_1^2+S_2^2+S_3^2$	$l_0(S_0+2S_1)$	$m_0(S_0+2S_2)$	$n_0(S_0+2S_3)$
AbC	$S_2-S_2^2+2S_1S_3$	$l_2(S_0+2S_1)$	m_2S_0	$n_2(S_0+2S_3)$
ABc	$S_3-S_3^2+2S_1S_2$	$l_3(S_0+2S_1)$	$m_3(S_0+2S_2)$	n_3S_0
Abc	$S_1^2+2S_1S_0$	$l_1(S_0+2S_1)$	m_1S_0	n_1S_0
aBC	$S_1-S_1^2+2S_2S_3$	l_1S_0	$m_1(S_0+2S_2)$	$n_1(S_0+2S_3)$
abC	$S_3^2+2S_3S_0$	l_3S_0	m_3S_0	$n_3(S_0+2S_3)$
aBc	$S_2^2+2S_2S_0$	l_2S_0	$m_2(S_0+2S_2)$	n_2S_0
abc	S_0^2	l_0S_0	m_0S_0	n_0S_0

The l's in the column for $\partial\pi/\partial y_1$ are $-\frac{1}{2}$ or $\frac{1}{2}$ according as the factors other than the first in the representation of the corresponding

phenotypes form old or new combinations. These can be readily obtained from table 3 by replacing unity by $\frac{1}{2}$. The appropriate scores, which are used in the calculation of efficient scores, for any class, are calculated by dividing the derivatives by the class probability. The information matrix is evaluated by the formulæ

$$I_{rr} = nS\left(\frac{1}{\pi}\frac{\partial\pi}{\partial y_r}\right)\frac{\partial\pi}{\partial y_r}$$

$$I_{rk} = nS\left(\frac{1}{\pi}\frac{\partial\pi}{\partial y_r}\right)\frac{\partial\pi}{\partial y_k} = nS\,\frac{\partial\pi}{\partial y_r}\left(\frac{1}{\pi}\frac{\partial\pi}{\partial y_k}\right)$$

where n is the total of observed frequencies. The maximum likelihood estimates, in this case, are obtained by successive approximations as explained in the next section.

4. MAXIMUM LIKELIHOOD ESTIMATES OF THE RECOMBINATION FRACTIONS TAKEN AS FREE PARAMETERS

When the data from various sources and different types of crosses giving information on only one or all the three recombination fractions are available, there arise the problems of obtaining the best estimates from the combined data and testing homogeneity of different parts of the data. The numerical computation of the above problems can be arranged as follows. To start with the scores and the information matrix, at the approximate values of y_1, y_2, y_3 are calculated for separate portions of the data as shown in table 5.

TABLE 5

Efficient scores and information matrix at the approximate values

Source and type of cross	Efficient scores			Information matrix					
1	$\phi_1^{(1)}$	$\phi_2^{(1)}$	$\phi_3^{(1)}$	$I_{11}^{(1)}$	$I_{12}^{(1)}$	$I_{13}^{(1)}$	$I_{22}^{(1)}$	$I_{23}^{(1)}$	$I_{33}^{(1)}$
.	.	.	.	.	.	.	.	.	.
k	$\phi_1^{(k)}$	$\phi_2^{(k)}$	$\phi_3^{(k)}$	$I_{11}^{(k)}$	$I_{12}^{(k)}$	$I_{13}^{(k)}$	$I_{22}^{(k)}$	$I_{23}^{(k)}$	$I_{33}^{(k)}$
Total	Φ_1	Φ_2	Φ_3	T_{11}	T_{12}	T_{13}	T_{22}	T_{23}	T_{33}

In the above table, if any part of the data gives information for only one segment, say the first, then only ϕ_3 and I_{33} are present and the rest are zero for that part. The methods of scoring in such cases have been fully discussed by Fisher (1946) and Bhat (1947) and also illustrated in this article in section 7 (*c*). For scoring parts of the data giving the simultaneous segregation of three factors the expressions derived in section 3 may be used.

Using the totals of table 5, the three linear equations in dy_1, dy_2 and dy_3, which are additive corrections to the first approximations of y_1, y_2 and y_3 respectively, can be written

$$\begin{aligned} T_{11}\,dy_1 + T_{12}\,dy_2 + T_{13}\,dy_3 &= \Phi_1 \\ T_{12}\,dy_1 + T_{22}\,dy_2 + T_{23}\,dy_3 &= \Phi_2 \\ T_{13}\,dy_1 + T_{23}\,dy_2 + T_{33}\,dy_3 &= \Phi_3 \end{aligned}$$

These calculations require to be repeated until the values of y_1, y_2 and y_3 for which the total scores are negligible are obtained.

If the scores and information matrix for the i-th part of the data at the estimated values are represented by

$$\dot{\phi}_1^{(i)},\ \dot{\phi}_2^{(i)},\ \dot{\phi}_3^{(i)} \text{ and } (\dot{I}_{rs}^{(i)})$$

then using the elements of the matrix $(\dot{I}_{(i)}^{rs})$ reciprocal to $(\dot{I}_{rs}^{(i)})$ a χ^2 can be calculated by the formula

$$\chi_i^2 = \text{SS}\, \dot{I}_{(i)}^{rs}\, \dot{\phi}_r^{(i)}\, \dot{\phi}_s^{(i)}$$

If the i-th part of the data concerns only two of the factors, say the first and second, then χ_i^2 is simply $[\dot{\phi}_3^{(i)}]^2/\dot{I}_{33}^{(i)}$. To test for heterogeneity the total

$$\chi^2 = \chi_1^2 + \ \ldots\ + \chi_k^2$$

can be used as χ^2 with degrees of freedom

$$d_1 + \ \ldots\ + d_k - 3$$

where $d_i = 1$ or 3 according as the i-th part of the data relates to the segregation of only two or three factors. A significant χ^2 at a chosen probability level indicates that different parts of the data are not homogeneous.

5. MAXIMUM LIKELIHOOD ESTIMATES SUBJECT TO KOSAMBI'S FORMULA

The general formula $\tanh 2x = 2y$ giving the relation between the map distance x and the recombination fraction y is given by Kosambi (1944). In terms of y_1, y_2 and y_3, the relation becomes

$$y_2 = \frac{y_1 + y_3}{1 + 4y_1y_3}$$

There are only two parameters y_1 and y_3 to be estimated, the third one, y_2, being obtainable from the above formula. The scores and information matrix for y_1 and y_3 can be conveniently calculated from the corresponding expressions for y_1, y_2 and y_3 taken as free parameters and in any practical problem the data may be scored for y_1, y_2 and y_3 taken as free parameters and then the appropriate scores for y_1 and y_3, when Kosambi's formula is applicable, can be deduced. If ψ_1 and ψ_3 represent the total scores for y_1 and y_3 and J_{11}, J_{13}, J_{33}, the elements of the information matrix then the formulæ connecting them with the totals of table 5 are

$$\psi_1 = \Phi_1 + \mu_1\Phi_2 \quad , \quad \mu_1 = (1 - 4y_3^2)/(1 + 4y_1y_3)^2$$
$$\psi_3 = \Phi_3 + \mu_3\Phi_2 \quad , \quad \mu_3 = (1 - 4y_1^2)/(1 + 4y_1y_3)^2$$

$$J_{11} = T_{11} + 2\mu_1T_{12} + \mu_1^2T_{22}$$
$$J_{13} = T_{13} + \mu_1T_{23} + \mu_3T_{12} + \mu_1\mu_3T_{22}$$
$$J_{33} = T_{33} + 2\mu_3T_{23} + \mu_3^2T_{22}$$

The additive corrections dy_1 and dy_3 to approximate values of y_1 and y_3 are obtained from the equations

$$J_{11}\ dy_1+J_{13}\ dy_3=\psi_1$$
$$J_{13}\ dy_1+J_{33}\ dy_3=\psi_3$$

If $\ddot{\Phi}_2$ is the total score for y_2 and $(\ddot{T}^{rs})$ is the inverse of the total information matrix calculated as in table 5 for y_1, y_2, y_3 at the estimated values $\ddot{y}_1, \ddot{y}_2, \ddot{y}_3$, subject to Kosambi's formula, then

$$\chi^2=\ddot{\Phi}_2^2(\ddot{T}^{22}+\mu_1^2\ddot{T}^{11}+\mu_3^2\ddot{T}^{33}+2\mu_1\mu_3\ddot{T}^{13}-2\mu_1\ddot{T}^{12}-2\mu_3\ddot{T}^{23})$$

can be used as χ^2 with 1 d.f. to test the agreement with Kosambi's formula. The estimates, obtained subject to Kosambi's formula, are valid only when the above test does not show significance.

6. AN ILLUSTRATIVE EXAMPLE

The methods developed in sections 3, 4 and 5 are applied to the linkage data for *Primula sinensis* reproduced from a paper by de Winton and Haldane (1935).

The data chosen consist of two types of back crosses so that exact values of maximum likelihood estimates could be obtained. The method of scoring can also be applied, starting with some trial values, and the first approximations which, in this case, must be identical with the exact values may be obtained.

The trial values chosen are $y_1=\cdot35$, $y_2=\cdot39$, $y_3=\cdot07$ so that,

$$\lambda_0=1/(2-y_1-y_2-y_3)=1/1\cdot19=\cdot840336$$
$$\lambda_1=1/(y_2+y_3-y_1)=1/\cdot11=9\cdot090909$$
$$\lambda_2=1/(y_1+y_3-y_2)=1/\cdot03=33\cdot333333$$
$$\lambda_3=1/(y_1+y_2-y_3)=1/\cdot67=1\cdot492537$$

The λ's are one-fourth of the reciprocals of the S-functions so that for any back cross the scores given in table 2 are obtained by multiplying the λ's with the appropriate values of the l's, m's and n's given in table 3.

TABLE 6

Linkage data of SBL in Primula sinensis (♀ *side*)

Set I $\dfrac{\text{sbl}}{\text{sbl}}\times\dfrac{\text{SBL}}{\text{sbl}}$

Phenotype	Observed frequency	Type of cross over	Scores for y_1	y_2	y_3	Multiplier
SBL	457	(0)	−	−	−	$\cdot840336=1/4S_0$
SbL	11	(12)	+	−	+	$33\cdot333333=1/4S_2$
SBl	256	(2)	+	+	−	$1\cdot492537=1/4S_3$
Sbl	38	(1)	−	+	+	$9\cdot090909=1/4S_1$
sBL	45	(1)	−	+	+	$9\cdot090909=1/4S_1$
sbL	284	(2)	+	+	−	$1\cdot492537=1/4S_3$
sBl	20	(12)	+	−	+	$33\cdot333333=1/4S_2$
sbl	469	(0)	−	−	−	$\cdot840336=1/4S_0$
	1580					

Set II $\frac{sbl}{sbl} \times \frac{SBl}{sbL}$

Phenotype	Observed frequency	Type of cross over	Scores for y_1	y_2	y_3	Multiplier
SBL	21	(2)	+	+	−	1·492537 = 1/4S$_0$
SbL	3	(1)	−	+	+	9·090909 = 1/4S$_2$
SBl	50	(0)	−	−	−	·840336 = 1/4S$_3$
Sbl	1	(12)	+	−	+	33·333333 = 1/4S$_1$
sBL	1	(12)	+	−	+	33·333333 = 1/4S$_3$
sbL	57	(0)	−	−	−	·840336 = 1/4S$_3$
sBl	4	(1)	−	+	+	9·090909 = 1/4S$_2$
sbl	26	(2)	+	+	−	1·492537 = 1/4S$_0$
	163					

The exact values from the combined data are given by

$$\dot{y}_1 = \frac{(2)+(12)}{N} = \frac{(256+284+11+20)+(21+1+1+26)}{1580+163}$$
$$= \frac{620}{1743} = \cdot 355709$$
$$\dot{y}_2 = \frac{(1)+(2)}{N} = \frac{677}{1743} = \cdot 388411$$
$$\dot{y}_3 = \frac{(1)+(12)}{N} = \frac{123}{1743} = \cdot 070568$$

The efficient scores at the trial values.

Set I	306·606720	−250·969032	203·757654
Set II	−16·736410	−22·797016	−29·762162
Total =	289·870310	−273·766048	173·995492

If the data contained some parts giving only two factors segregations then the scores arising from them have to be added to the above values. Such data can supply only one of the efficient scores, the others being considered as zero. In such cases the appropriate scores for intercrosses are given on p. 61 and for back crosses on p. 58 in Mather's book, *The Measurement of Linkage in Heredity* (first edition).

The information matrix per single observation is the same for both the above sets at the trial values.

$$i_{11} = i_{22} = i_{33} = \tfrac{1}{2}(\lambda_0+\lambda_1+\lambda_2+\lambda_3) = \quad 22\cdot 378557$$
$$i_{12} = \tfrac{1}{2}(\lambda_0-\lambda_1-\lambda_2+\lambda_3) = -20\cdot 045685$$
$$i_{23} = \tfrac{1}{2}(\lambda_0+\lambda_1-\lambda_2-\lambda_3) = -12\cdot 447312$$
$$i_{13} = \tfrac{1}{2}(\lambda_0-\lambda_1+\lambda_2-\lambda_3) = \quad 11\cdot 795111$$

The total information matrix **T** is

$$\mathbf{T} = (1580+163)\begin{pmatrix} 22\cdot 378557 & -20\cdot 045685 & 11\cdot 795111 \\ -20\cdot 045685 & 22\cdot 378557 & -12\cdot 447312 \\ 11\cdot 795111 & -12\cdot 447312 & 22\cdot 378557 \end{pmatrix}$$

and its inverse is

$$\mathbf{T}^{-1} = \frac{1}{1743}\begin{pmatrix} \cdot 227500 & \cdot 198500 & -\cdot 009500 \\ \cdot 198500 & \cdot 237900 & \cdot 027700 \\ -\cdot 009500 & \cdot 027700 & \cdot 065100 \end{pmatrix}$$

The corrections to trial values are given by

$$dy_i = \Phi_1 T^{i1} + \Phi_2 T^{i2} + \Phi_3 T^{i3}$$

$$dy_1 = \frac{1}{1743}[\cdot 2275(289\cdot 870310) + \cdot 1985(-273\cdot 766048) - \cdot 0095(173\cdot 995492)] = \cdot 00570953$$

$$dy_2 = \frac{1}{1743}[\cdot 1985(\quad) + \cdot 2379(\quad) + \cdot 0277(\quad)] = -\cdot 00158922$$

$$dy_3 = \frac{1}{1743}[-\cdot 0095(\quad) + \cdot 0277(\quad) + \cdot 0651(\quad)] = \cdot 000567990$$

The first approximations to trial values ·35, ·39, ·07, are

·355709, ·388411, ·070568

agreeing accurately up to the number of significant figures maintained in the calculations with the exact estimates

·355709, ·388411, ·070568

obtained earlier. This is not generally true but if the trial values of the cross over percentages can be correctly guessed to the nearest whole numbers the first approximations obtained by the method of scoring are expected to be sufficiently accurate.

The test of the hypothesis that the two sets of data arise from identical values of recombination fractions involves the evaluation of the scores and information matrices at the estimated values. The new scores can, however, be obtained approximately by certain adjustments of the scores at the trial values. The change in the matrix inverse to the information matrix is negligible so that no adjustment is necessary. But in cases where approximations differ considerably from the trial values it is necessary to calculate the scores and information matrix directly.

The adjusted scores for the first set are

$$\dot{\phi}_1^{(1)} = \phi_1^{(1)} - n_1(dy_1 i_{11} + dy_2 i_{12} + dy_3 i_{13})$$
$$= 289\cdot 870310 - 1580[22\cdot 378557(\cdot 005709) - 20\cdot 045685(-\cdot 001589) + 11\cdot 795111(\cdot 000568)]$$
$$= 289\cdot 870310 - 262\cdot 771854 = 27\cdot 098456$$

$$\dot{\phi}_2^{(1)} = -273\cdot 766048 - 1580[-20\cdot 045685(\quad) + 22\cdot 378557(\quad) - 12\cdot 447312(\quad)]$$
$$= -273\cdot 766048 + 248\cdot 171256 = -25\cdot 594792$$

$$\dot{\phi}_3^{(1)} = 173\cdot 995492 - 1580[11\cdot 795111(\quad) - 12\cdot 447312(\quad) + 22\cdot 378557(\quad)]$$
$$= 173\cdot 995492 - 157\cdot 728377 = 16\cdot 267115$$

The value of χ_1^2 is,

$$= \frac{1}{n_1} SS_{rs}\dot{\phi}_r^{(1)}\dot{\phi}_s^{(1)}$$

$$= \frac{1}{1580}[\cdot 2275(27\cdot 0984)^2 + \cdot 2379(25\cdot 5948)^2 + \cdot 0651(16\cdot 2671)^2$$
$$+2(\cdot 1985)(27\cdot 0984)(-25\cdot 5948)$$
$$+2(-\cdot 0095)(\quad)(16\cdot 2671)$$
$$+2(\cdot 0277)(-25\cdot 5948)(\quad)]$$

$$= \frac{33\cdot 3807}{1580} = \cdot 02113.$$

Similarly

$\dot{\phi}_1^{(2)} = -27\cdot 098456$, $\dot{\phi}_2^{(2)} = 25\cdot 594792$, $\dot{\phi}_3^{(2)} = -16\cdot 267115$ and

$$\chi_2^2 = \frac{33\cdot 3807}{163} = \cdot 2048.$$

The total $\chi^2 = \chi_1^2 + \chi_2^2 = \cdot 0211 + \cdot 2048 = \cdot 2259$ is considerably smaller than its expectation $3+3-3 = 3$, the degrees of freedom. The two sets of data may be regarded as homogeneous.

The variances of the estimates from the combined data are given by the diagonal elements of the matrix $\mathbf{T}^{-1}$.

$$V(\dot{\gamma}_1) = \cdot 2275/1743 = 10^{-3}(\cdot 130522)$$
$$V(\dot{\gamma}_2) = \cdot 2379/1743 = 10^{-3}(\cdot 136489)$$
$$V(\dot{\gamma}_3) = \cdot 0651/1743 = 10^{-3}(\cdot 037349)$$

These are only approximate values, the exact values being obtainable from the inverse of the information matrix calculated at the estimated values.

If Kosambi's formula is assumed, there are only two parameters γ_1 and γ_3 to be estimated. The appropriate scores and information matrix in this case are calculated from the values obtained before for γ_1, γ_2 and γ_3 considering them as free parameters. The trial values of γ_1, γ_2 and γ_3 are the same as before. To start with calculate

$$\mu_1 = \frac{1-4\gamma_3^2}{(1+4\gamma_1\gamma_3)^2}, \quad \mu_3 = \frac{1-4\gamma_1^2}{(1+4\gamma_1\gamma_3)^2}$$
$$= \frac{\cdot 980400}{1\cdot 205604}, \quad = \frac{\cdot 510000}{1\cdot 205604}$$
$$= \cdot 813202, \quad = \cdot 423024$$
$$\mu_1^2 = \cdot 661297, \quad \mu_1\mu_3 = \cdot 344004, \quad \mu_3^2 = \cdot 178949.$$

The efficient scores ψ_1 and ψ_3 are,

$$\psi_1 = \Phi_1 + \mu_1\Phi_2$$
$$= 289\cdot 870310 + \cdot 813202(-273\cdot 766048)$$
$$= 67\cdot 243211$$
$$\psi_3 = \Phi_3 + \mu_3\Phi_2$$
$$= 173\cdot 995492 + \cdot 423024(-273\cdot 766048)$$
$$= 58\cdot 185880$$

Information matrix for y_1, y_3.

$j_{rs}=$	j_{11}	j_{13}	j_{33}
$+t_{rs}$	22·378557	11·795111	22·378557
	t_{11}	t_{13}	t_{33}
$+\mu_r\mu_s t_{22}$	14·798873	7·698313	4·004620
	$\mu_1^2 t_{22}$	$\mu_1\mu_3 t_{22}$	$\mu_3^2 t_{22}$
$+\mu_r t_{s2}$	−16·301191	−8·479806	−5·265512
	$\mu_1 t_{12}$	$\mu_3 t_{12}$	$\mu_3 t_{23}$
$+\mu_s t_{r2}$	−16·301191	−10·122179	−5·265512
	$\mu_1 t_{12}$	$\mu_1 t_{23}$	$\mu_3 t_{23}$
Total	4·575048	·891439	15·852153

$$J = 1743\begin{pmatrix} 4\cdot575048 & \cdot891439 \\ \cdot891439 & 15\cdot852153 \end{pmatrix}$$

$$J^{-1} = \frac{1}{1743}\begin{pmatrix} \cdot220998 & -\cdot012428 \\ -\cdot012428 & \cdot063782 \end{pmatrix}$$

The additive corrections to y_1 and y_3 are given by

$$dy_1 = \frac{1}{1743}[\cdot220998(67\cdot243211) - \cdot012428(58\cdot185880)]$$
$$= 14\cdot137481/1743 = \cdot00811100$$
$$dy_3 = \frac{1}{1743}[-\cdot012428(\quad) + \cdot063782(\quad)]$$
$$= 2\cdot875513/1743 = \cdot000164975$$

so that the estimates of y_1 and y_3 are

$$\cdot358111 \text{ and } \cdot070165$$

and the estimate of y_2 as obtained from the formula is

$$\ddot{y}_2 = (\ddot{y}_1+\ddot{y}_3)/(1+4\ddot{y}_1\ddot{y}_3)$$
$$= (\cdot428276)/1\cdot100507 = \cdot389162$$

which differ very slightly from the estimates obtained by treating the y's as free parameters. The goodness of fit of Kosambi's formula to the cross over values indicated by the data may be tested as follows.

This needs the evaluation of the total efficient score $\ddot{\Phi}_2$ at these estimated values. As before, a good approximation to this value can be obtained by using the formula

$$\ddot{\Phi}_2 = \Phi_2 - N[i_{21}(\ddot{y}_1-\cdot35)+i_{22}(\ddot{y}_2-\cdot39)+i_{23}(\ddot{y}_3-\cdot07)$$
$$= -273\cdot766048 - 1743[-20\cdot045685(\cdot008111)$$
$$+22\cdot378557(-\cdot000838) - 12\cdot447312(\cdot000164975)]$$
$$= -273\cdot766048+319\cdot661912$$
$$= \quad 45\cdot895864$$

$$1/V(\ddot{\Phi}_2) = \frac{1}{N}[i^{22}+\mu_1^2 i^{11}+\mu_3^2 i^{33}+2\mu_1\mu_3 i^{13}-2\mu_1 i^{12}-2\mu_3 i^{23}]$$
$$= 10^{-4}(\cdot270694).$$

The quantity

$$\chi^2 = (\ddot{\Phi}_2)^2/V(\ddot{\Phi}_2) = (45{\cdot}895864)^2 \times 10^{-4}({\cdot}270694) = {\cdot}0570$$

can be used as χ^2 with one degree of freedom to test Kosambi's formula. The observed χ^2 is very small, thus indicating fair agreement with Kosambi's formula.

The variances of the estimates obtained by using Kosambi's formula are,

$$V(\ddot{y}_1) = \frac{j^{11}}{N} = \frac{{\cdot}220998}{1743} = 10^{-3}({\cdot}126791)$$

$$V(\ddot{y}_3) = \frac{j^{33}}{N} = \frac{{\cdot}063782}{1743} = 10^{-3}({\cdot}036593)$$

$$V(\ddot{y}_2) = \frac{\mu_1^2 j^{11} + 2\mu_1\mu_3 j^{13} + \mu_3^2 j^{33}}{N} = \frac{{\cdot}149008}{1743} = 10^{-3}({\cdot}085489).$$

By comparing these variances with those obtained before, we find the percentage increase in efficiency by using Kosambi's formula as

$$2{\cdot}94,\ 59{\cdot}66,\ 2{\cdot}06$$

for y_1, y_2 and y_3 respectively.

7. THE INCREASE IN PRECISION BY THE USE OF EFFICIENT SCORES

In the previous sections, methods have been developed for the appropriate scoring of data relating to the simultaneous segregation of three factors. It is, however, of importance to calculate the gain in efficiency by following this method instead of replacing the data by three marginal distributions obtained by ignoring one factor each time and treating the distributions as independent. In such a case the data can be scored by considering only one segment at a time. It is seen in section 3 that this makes no difference in the case of back crosses. The general investigation of this problem in the case of F_2 intercrosses is difficult but an example may be considered to give an idea of the increase in efficiency.

Let the data consist of the results of the F_2 of $\frac{AbC}{aBc}$ classified in eight phenotypical classes. The variances and covariances of the estimates, from the data of the above type of y_1, y_2, y_3 considered as (1) free parameters and (2) subject to Kosambi's formula, can be calculated in each case by using (*a*) the method of efficient scores and (*b*) the individual segment method, and the relative efficiency of the method (*b*) in each case can be found out. The following are the calculations in the particular case wherein the recombination fractions are

$$y_3 = {\cdot}03,\ y_1 = {\cdot}28 \text{ and } y_2 = {\cdot}30.$$

(a) Method of efficient scores taking y's as free parameters

Using table 4, the appropriate S-functions for the triple heterozygote AbC/aBc are calculated at the above chosen values.

$$\begin{aligned} S_3 &= \tfrac{1}{4}(y_2+y_3-y_1) &&= \cdot 0125 \\ S_2 &= \tfrac{1}{4}(2-y_3-y_2-y_1) &&= \cdot 3475 \\ S_1 &= \tfrac{1}{4}(y_2+y_1-y_3) &&= \cdot 1375 \\ S_0 &= \tfrac{1}{4}(y_3+y_1-y_2) &&= \cdot 0025 \end{aligned}$$

$\cdot 5000$ (check)

The values of the l's, m's, n's of table 3 with 1 replaced by $\frac{1}{2}$ are

$$l_2 = l_3 = m_2 = m_0 = n_2 = n_1 = -\tfrac{1}{2}$$
$$l_1 = l_0 = m_1 = m_3 = n_0 = n_3 = \tfrac{1}{2}$$

The probabilities, derivatives and scores are given in table 7.

TABLE 7

Probabilities and scores

Phenotype	Probability 16π	Derivatives and scores					
		$16\frac{\partial \pi}{\partial y_1}$	$\frac{1}{\pi}\frac{\partial \pi}{\partial y_1}$	$16\frac{\partial \pi}{\partial y_2}$	$\frac{1}{\pi}\frac{\partial \pi}{\partial y_2}$	$16\frac{\partial \pi}{\partial y_3}$	$\frac{1}{\pi}\frac{\partial \pi}{\partial y_3}$
ABC	6·2771	2·22	·353666	−6·38	−·888944	·22	·025048
AbC	3·6829	−2·22	−·602788	−·02	−·005430	−·22	−·029868
ABc	1·7265	−2·22	−1·285838	6·38	3·231970	·02	·011584
Abc	·3135	2·22	7·081338	·02	·637960	−·02	−·637960
aBC	2·0365	·02	·009820	6·38	2·739920	−·22	−·108028
abC	·0035	−·02	−5·714286	·02	5·714286	·22	62·857140
aBc	1·9599	−·02	−·010204	−6·38	−2·847084	−·02	−·018204
abc	·0001	·02	200·000000	−·02	−200·000000	·02	200·000000
	16·0000	0·00		0·00		0·00	

The information matrix **i**, per single observation, can be calculated by using the formulæ of section 3 (*b*). Thus

$$i_{11} = \frac{1}{16}[2\cdot 22(\ \cdot 353666)+\ldots+\cdot 02(\ 200\cdot 000000)] = 1\cdot 550821,$$

$$i_{12} = \frac{1}{16}[2\cdot 22(-\cdot 888944)+\ldots+\cdot 02(-200\cdot 000000)] = -\cdot 812331,$$

etc.

$$\mathbf{i} = \begin{pmatrix} 1\cdot 550821 & -\cdot 812331 & \cdot 173998 \\ -\cdot 812331 & 3\cdot 642891 & -\cdot 213733 \\ \cdot 173998 & -\cdot 213733 & 1\cdot 117181 \end{pmatrix}$$

The covariance matrix **v**, per single observation, of the maximum likelihood estimates obtained by choosing such values of y_1, y_2, y_3

which make the scores for an observed set of frequencies vanish, is the inverse of the information matrix.

$$\mathbf{v} = \begin{pmatrix} \cdot 737882 & \cdot 159589 & -\cdot 084391 \\ \cdot 159589 & \cdot 312139 & \cdot 034861 \\ -\cdot 084391 & \cdot 034861 & \cdot 914923 \end{pmatrix}$$

(b) Method of efficient scores : y's being subject to Kosambi's formula

The information matrix j, per single observation, for y_1, y_3 in this case is obtained from i, calculated above, by using the formula given in section 5 and illustrated in section 6.

The procedure of computation is as follows :—

$$\mu_1 = \cdot 932671 \quad , \quad \mu_3 = \cdot 642498$$

j_{rs}	j_{11}	j_{13}	j_{33}
i_{rs}	1·550821	·173998	1·117181
$\mu_r\mu_s i_{22}$	3·168852	2·182996	1·503807
$\mu_r i_{s2}$	−·757637	−·521921	−·137323
$\mu_s i_{2r}$	−·757637	−·199342	−·137323
Total	3·204399	1·635701	2·346342

The covariance matrix u, per single observation, of the estimates of y_1 and y_3 is the inverse of j.

$$\mathbf{u} = \begin{pmatrix} \cdot 484471 & -\cdot 337738 \\ -\cdot 337738 & \cdot 661642 \end{pmatrix}$$

If $\ddot{y}_1$ and $\ddot{y}_3$ are the estimates of y_1 and y_3 then, substituting these values in Kosambi's formula, we get $\ddot{y}_2$, the estimate of y_2. The variance of $\ddot{y}_2$ is calculated by the formula,

$$V(\ddot{y}_2) = \cdot 484471(\cdot 932671)^2 - 2(\cdot 337738)(\cdot 932671)(\cdot 642498) + \cdot 661642(\cdot 642498)^2$$

$$= \cdot 289786 \text{ per single observation.}$$

(c) Individual segment method taking y's as free

In the individual segment method, only two factors are considered each time and the data are scored for the corresponding recombination fraction. The results of the cross AbC × aBc supply data in repulsion for the estimation of y_3 and y_1 and in coupling for y_2. To score for y_1, it is necessary to consider the scores appropriate to data in repulsion as given in Mather's book, *The Measurement of Linkage in Heredity*, p. 61 (first edition). The observed frequencies in the eight phenotypical classes are represented by n_{ijk} as in table 2. Ignoring the classification with respect to the first factor we get the scores at $y_1 = \cdot 28$

in the four phenotypical classes and observed frequencies as given in table 8.

TABLE 8

Scores in repulsion at $y_1 = \cdot 28$

Phenotype	Observed frequency, ignoring the first factor	Scores
BC	$n_{111}+n_{011}$	·269438
Bc	$n_{110}+n_{010}$	−·607639
bC	$n_{101}+n_{001}$	−·607639
bc	$n_{100}+n_{000}$	7·142857

If the total score for y_1 by this method is represented by Q_1, then

$$Q_1 = \cdot 269438(n_{111}+n_{011}) - \cdot 607639(n_{110}+n_{010}+n_{101}+n_{001}) + 7\cdot 142857(n_{100}+n_{000})$$

The scores for y_2 and y_3 are similarly calculated.

$$Q_2 = -\cdot 562249(n_{111}+n_{101}) + 2\cdot 745098(n_{110}+n_{100}+n_{011}+n_{001}) - 2\cdot 857143(n_{010}+n_{000})$$

$$Q_3 = \cdot 029986(n_{111}+n_{110}) - \cdot 060054(n_{101}+n_{100}+n_{011}+n_{010}) + 66\cdot 666667(n_{001}+n_{000})$$

The estimate of y_i is found, by this method, by choosing that value of y_i which makes the score Q_i zero for the observed set of frequencies. The estimates differ from the true values unless the frequencies are the same as their expected values. If the probabilities of given departures of the Q's from their expected values or simply their sampling errors in large samples are known, then the corresponding errors introduced in the estimate can be calculated. Since the Q's are linear functions of the frequencies in the eight phenotypical classes with probabilities as given in table 7, the covariance m_{ij}, per single observation, between Q_i and Q_j can be calculated (as given in Fisher's *Statistical Methods*, p. 303, ninth edition) by taking the triple products of coefficients of the frequency for any class in Q_i and Q_j and the probability for that class and summing over all the eight classes. Thus

$$m_{11} = \frac{1}{16}[(\cdot 269438)^2(6\cdot 2771+2\cdot 0365)+(\cdot 607639)^2(1\cdot 7265+1\cdot 9599 + 3\cdot 6829+\cdot 0035)+(7\cdot 142857)^2(\cdot 3135+\cdot 0001)]$$
$$= 1\cdot 207860.$$

The matrix $\mathbf{m} = (m_{ij})$, thus calculated, comes out as

$$\mathbf{m} = \begin{pmatrix} 1\cdot 270860 & \cdot 526986 & -\cdot 002269 \\ \cdot 526986 & 3\cdot 118356 & \cdot 046039 \\ -\cdot 002269 & \cdot 046039 & 1\cdot 002248 \end{pmatrix}$$

The covariance of the estimates of y_i and y_j is given by $m_{ij}/m_{ii}m_{jj}$

so that, using the above elements, we can set up the covariance matrix $\mathbf{v}^*$ of the estimates of y_1, y_2, y_3.

$$\mathbf{v}^* = \begin{pmatrix} \cdot 827907 & \cdot 139913 & -\cdot 001874 \\ \cdot 139913 & \cdot 320680 & \cdot 014731 \\ -\cdot 001874 & \cdot 014731 & \cdot 997804 \end{pmatrix}$$

(d) Individual segment method, y's being subject to Kosambi's formula

In this there are two parameters, y_1 and y_3, to be estimated. The scores P_1, P_3 for y_1 and y_3 by this method are,

$$P_1 = Q_1 + \mu_1 Q_2$$
$$P_3 = Q_3 + \mu_3 Q_2$$

so that, knowing Q_1, Q_2, Q_3 considered above, and the differential coefficients μ_1, μ_3, the P's can be calculated. As the covariances of the Q's are known the covariances of P's can be calculated as follows

$$\begin{aligned} V(P_1) &= V(Q_1) + \mu_1^2 V(Q_2) + 2\mu_1 \text{ Cov } (Q_1 Q_2) \\ &= 1 \cdot 207860 + (\cdot 932671)^2 (3 \cdot 118356) + 2(\cdot 932671)(\cdot 526986) \\ &= 4 \cdot 903438. \end{aligned}$$

$$\begin{aligned} \text{Cov } (P_1 P_3) &= \text{Cov } (Q_1 Q_3) + \mu_1 \text{ Cov } (Q_2 Q_3) + \mu_3 \text{ Cov } (Q_1 Q_2) \\ &\qquad + \mu_1 \mu_3 V(Q_2) \\ &= 2 \cdot 247897. \end{aligned}$$

Similarly

$$V(P_3) = 2 \cdot 348684.$$

Thus $\mathbf{d}$, the covariance matrix, per single observation, of P_1, P_3 is

$$\mathbf{d} = \begin{pmatrix} 4 \cdot 903438 & 2 \cdot 247897 \\ 2 \cdot 247897 & 2 \cdot 348684 \end{pmatrix}$$

If the Q's are independent, *i.e.* if three sets of data containing the same number of observations as the total in the case were independently observed for the three segments separately, then the individual segment method is the most efficient method. In this case the covariance matrix $\mathbf{a}$, per single observation, of P_1, P_3 is obtained from the above calculations by dropping the covariance terms. Thus

$$\begin{aligned} V(P_1) &= 1 \cdot 207860 + (\cdot 932671)^2 (3 \cdot 118356) \\ &= 3 \cdot 920430 \end{aligned}$$

and

$$\mathbf{a} = \begin{pmatrix} 3 \cdot 920430 & 1 \cdot 868640 \\ 1 \cdot 868640 & 2 \cdot 289524 \end{pmatrix}$$

Its reciprocal $\mathbf{a}^{-1}$ gives, when the Q's are independent, the covariance matrix of the estimates obtained by choosing values of y_1 and y_3 which make the scores P_1 and P_3 vanish. This, on calculation, comes out as

$$\mathbf{a}^{-1} = \begin{pmatrix} \cdot 417483 & -\cdot 340737 \\ -\cdot 340737 & \cdot 714871 \end{pmatrix}$$

When the Q's are not independent the estimates, obtained by choosing such values of y_1 and y_3 which make the scores P_1 and P_3 for the observed set of frequencies vanish, have the covariance matrix $\mathbf{u}^*$ given by

$$\mathbf{u}^* = \mathbf{a}^{-1}\mathbf{d}\mathbf{a}^{-1}$$

This is a triple product of the matrices $\mathbf{a}^{-1}$, $\mathbf{d}$ and $\mathbf{a}^{-1}$. To evaluate this the product $\mathbf{a}^{-1}\mathbf{d}$ may be found first and then multiplied by $\mathbf{a}^{-1}$. The method of multiplying two matrices is to construct a matrix whose element in the i-th row and the j-th column is the sum of products of the ordered elements in the i-th row of the first matrix and j-th column of the second matrix.

$$\begin{aligned}
\mathbf{u}^* &= \begin{pmatrix} \cdot417483 & -\cdot340737 \\ -\cdot340737 & \cdot714871 \end{pmatrix}\begin{pmatrix} 4\cdot903438 & 2\cdot247897 \\ 2\cdot247897 & 2\cdot348684 \end{pmatrix}\mathbf{a}^{-1} \\
&= \begin{pmatrix} 1\cdot281160 & \cdot138175 \\ -\cdot063827 & \cdot913064 \end{pmatrix}\begin{pmatrix} \cdot417483 & -\cdot340737 \\ -\cdot340737 & \cdot714871 \end{pmatrix} \\
&= \begin{pmatrix} \cdot487781 & -\cdot337761 \\ -\cdot337761 & \cdot674472 \end{pmatrix}
\end{aligned}$$

The variance, per single observation, of the estimate of y_2 as calculated from the formula given in section 7 (b) is

$$(\cdot487781)(\cdot932671)^2 - 2(\cdot337761)(\cdot932671)(\cdot642498) + (\cdot674472)(\cdot642498)^2 = \cdot297933$$

A comparison is made below of the covariance matrices $\mathbf{v}$, $\mathbf{v}^*$, $\mathbf{u}$, $\mathbf{u}^*$ of the estimates obtained by the four methods discussed above. Choosing the variances alone we have the comparisons as shown in table 9.

TABLE 9

Variances of estimates and relative efficiencies

Method	Variances of estimates of y's treated as						Percentage increase in efficiency due to Kosambi's formula		
	Free parameters			Subject to Kosambi's formula					
	y_1	y_2	y_3	y_1	y_2	y_3	y_1	y_2	y_3
(α) Efficient scores	·737882	·312139	·914923	·484471	·289786	·661642	52·30	7·71	38·28
(β) Individual segment	·827907	·320680	·997804	·487781	·297933	·674472	69·73	7·63	47·94
(γ) Relative efficiency $= \frac{(\alpha)}{(\beta)} \times 100$	89·12	97·34	91·69	99·32	97·31	98·10	...	...	...

A measure of overall efficiency can be obtained by comparing the covariance matrices of the estimates by the two methods. Instead

of the simple ratio of the determinants, its s-th root, where s is the number of parameters estimated, may be defined as the relative efficiency of one method as compared with another. Thus, when y's are considered as free parameters the overall efficiency of the individual segment method is

$$\left\{\frac{|\mathbf{v}|}{|\mathbf{v}^*|}\right\}^{\frac{1}{3}} = \left\{\frac{\cdot 183367}{\cdot 245190}\right\}^{\frac{1}{3}} = \sqrt[3]{\cdot 7478} = \cdot 9077 \text{ or } 90\cdot 77 \text{ per cent.}$$

The corresponding efficiency when y's are subject to Kosambi's formula is

$$\left\{\frac{|\mathbf{u}|}{|\mathbf{u}^*|}\right\}^{\frac{1}{3}} = \cdot 9801 \text{ or } 98\cdot 01 \text{ per cent.}$$

The overall increase in efficiency by using Kosambi's formula can be calculated by comparing the covariance determinants of the estimates of y_1 and y_3 alone. This increase for the method of efficient scores is

$$\frac{\begin{vmatrix} \cdot 737882 & \cdot 173998 \\ \cdot 173998 & \cdot 914923 \end{vmatrix}^{\frac{1}{2}}}{|\mathbf{u}|^{\frac{1}{3}}} - 1 = 79\cdot 89 \text{ per cent.}$$

and for the individual segment method

$$\frac{\begin{vmatrix} \cdot 827909 & -\cdot 001874 \\ -\cdot 001874 & \cdot 997804 \end{vmatrix}^{\frac{1}{2}}}{|\mathbf{u}^*|^{\frac{1}{3}}} - 1 = 96\cdot 06 \text{ per cent.}$$

These calculations lead to the following conclusions, (1) The use of Kosambi's formula, when applicable, considerably increases the efficiency of the estimates by either method. In the particular example chosen the overall increase in efficiency is 79·89 per cent. for the most efficient method and 96·06 per cent. for the individual segment method. (2) The loss of efficiency due to the simpler analysis of the individual segment method is smaller when the recombination fractions are estimated with the use of Kosambi's formula. In the above example the overall efficiency of the individual segment method, when y's are considered as free parameters, is 90·77 per cent. which increases to 98·01 per cent. when y's are subject to Kosambi's formula.

8. SUMMARY

The following results have been discussed in this article.

1. The appropriate scoring of data giving the simultaneous segregation of three factors and the method of arriving at the best estimates of recombination fractions from data relating to various sources and types of crosses have been discussed in the two cases (i) when they are taken as free parameters and (ii) when they conform to Kosambi's formula. It has been observed that the scores and information matrix

in the latter case are connected with those in the former by simple relations and in any practical example it is convenient to score the data considering the recombination fractions as free parameters and then deduce the total scores appropriate to the latter case.

It has been found, in the examples discussed in the article, that the estimates found by using Kosambi's formula have considerably smaller variances. This is so, for when this formula is true there are only two parameters to be estimated (the third one being deduced from the formula) and any method of estimation which does not make use of the formula, being different from the maximum likelihood method appropriate to the two parameters, is bound to be inefficient.

The use of such empirical relations as the one considered above among the parameters to be estimated, when known, enhances the precision of the estimates although they may not be strictly accurate. The assumption of a slightly inaccurate relationship may introduce bias in the estimates but such estimates are more useful than the less efficient estimates so long as the bias, in any case, is small in comparison with its standard error. This, in some way, is secured when the test for a hypothesis specifying some restrictions indicates close agreement with the observations. Kosambi's formula is very useful from this point of view, as its use considerably enhances the precision of the estimates. A test has been proposed to judge the validity of this formula in any particular case.

2. In view of the slightly heavier computation involved in the method of efficient scores an investigation has been made to find out the loss in efficiency due to the simpler method of scoring by considering the data as classified with respect to only two factors each time and considering them as independent distributions. The latter method is called the individual segment method.

It has been found that if the data consist of only back crosses both the methods lead to identical estimates, when the recombination fractions are considered as free parameters. This, however, is not true when the estimates are found subject to Kosambi's formula but the loss in efficiency is not expected to be considerable.

In the case of intercrosses a particular example has been chosen to find the relative efficiency of this method in the two cases when the recombination fractions are considered as (i) free parameters and (ii) subject to Kosambi's formula. It is found that the loss of information due to the simpler analysis is negligible when the recombination fractions conform to Kosambi's formula. This may not be generally true, but the loss in any case is not expected to be considerable. Similar results may be expected in the case of data giving the simultaneous segregation of more than three factors. Their exact treatment involves some complications because some extraneous parameters have to be estimated and accounted for in the evaluation of the variances of the estimates of the recombination fractions. This, however, awaits further study.

Finally, I should like to thank Professor R. A. Fisher for his guidance and criticism during the preparation of this paper and Dr K. Mather for his valuable suggestions which led to a clarification of the various steps involved in this article.

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COLOUR PATTERNS AND GENE FREQUENCIES IN NATURAL POPULATIONS OF A PLATYFISH *

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THE platyfish, *Platypoecilus maculatus* Günther, is one of the most highly polymorphic vertebrates in Central and North America, having over 150 recognisable colour patterns in its various natural populations. The black colour patterns are genetically controlled by two groups of genes. First, an autosomal series consisting of seven dominant alleles (Gordon, 1947) determines melanic patterns at or near the base of the tail fin ; these patterns are composed of micromelanophores. Second, a sex-linked series consisting of five dominant alleles (Gordon, 1948) produces another group of melanic patterns on the body proper ; these, however, are composed of macromelanophores, pigment cells which are much larger and more prominent than those which form the tail patterns.

According to a recent genetic and geographical analysis of the inheritance and distribution of the seven tail patterns, Gordon (1947) indicated that the four natural populations of the platyfish from the Rio Jamapa, Rio Papaloapan, Rio Coatzacoalcos and Rio Usumacinta are genetically distinct. In that analysis only single tail patterns were considered in comparing one population with another, but the frequencies of the dual tail pattern combinations were presented in the tabular matter.

The purpose of the present analysis is to determine whether the values observed for the seven single patterns are consistent with each other and with their various combinations. If they are, the observed frequency of a single pattern would correctly indicate the approximate frequency of the gene responsible for that pattern. Any conclusion drawn about the differences of the frequency of certain single patterns between two populations would also be true for the frequency of the genes corresponding to these patterns.

A careful analysis was made of the two largest populations, those of the Rio Jamapa and of Rio Papaloapan. No attempt was made at this time to calculate gene frequencies for the Rio Usumacinta and Rio Coatzacoalcos samples because the samples are small, considering

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the large number of possible patterns. Since there are no obvious inconsistencies in the observed numbers, however, it may be assumed that if the Rio Jamapa and Rio Papaloapan observations are consistent, the Rio Usumacinta and Rio Coatzacoalcos observations are also consistent.

METHOD

In the analysis of the Rio Jamapa platyfish population and in the first calculation in the study of the Rio Papaloapan population (see tables 1 and 2), the gene frequencies were found primarily by using the formula derived from Hardy (1908) :—

$$a = 1 - \sqrt{1-x}$$

where a is the gene frequency of the gene responsible for a pattern, and x the portion of the sample which shows that pattern both alone and in combination with another pattern. For each population, certain modifications of this method were needed, because some of the smaller tail patterns are concealed by the larger ones.

TABLE 1

Observed numbers, gene frequencies and calculated numbers based upon these gene frequencies for the various patterns in platyfish from the Rio Jamapa

Patterns	Observed nos.	Calculated nos.	Values for χ^2	Gene frequencies
+	57	59·9	0·14	+ 0·2641
O	277	305·6	2·68	*O* 0·3880
C	47	46·4	0·01	*C* 0·0875
Cc, *CCc*	89	68·5	6·13	*Cc* 0·0992
Co	44	44·1	0·00	*Co* 0·0838
T	54	40·3	5·06	*T* 0·0774
OC	62	58·3	0·23	...
OCc	69	66·2	0·11	...
OCo	72	55·9	4·63	...
OT	58	51·6	0·79	...
CCo	11	12·6	0·21	...
CT	9	11·6	0·59	...
CcCo	4	14·3	7·42	...
CcT	0	13·2	13·20	...
CoT	7	11·2	1·58	...
Total	860	859·7	42·84	1·0000

For example, *CCc* cannot be phenotypically distinguished from *Cc* alone, nor *MMc* from *Mc* (see fig. 1), so that the frequencies of *C* and *M* cannot be found by the formula. The frequency of *C* was found in each population by subtracting the frequency of *Cc* from that of *C* and *Cc* together. This latter frequency was first found by the formula indicated above.

In the Rio Papaloapan platyfish several patterns involving *C*, *Co*, or *T*, when combined with *M* are indistinguishable phenotypically from similar combinations with *Mc* (see fig. 1). The gene frequencies of

M and *Mc* in the population were found by first considering the fish with the doubtful patterns as neither *M* nor *Mc* and then making corrections for these doubtful individuals. The formulæ for these corrections may be derived as follows. Suppose that a portion *d* of

TABLE 2

Observed numbers, gene frequencies, and calculated numbers based upon these gene frequencies for the various patterns in platyfish from the Rio Papaloapan

Patterns	Obs. nos.	Calculated I	Additional	Obs. regrouped	Calculated II	Values for χ^2
+ . .	643	675·0	...	643	639·4	0·02
O . .	639	694·2	...	639	678·8	2·33
M . .	368	306·6	...	368	356·5	0·37
Mc, MMc .	40	31·1	*MT, McT*	42	41·2	0·02
C . .	158	150·9	...	158	146·7	0·87
Cc, CCc .	428	390·4	*MCc*	434	458·8	1·34
Co . .	276	298·2	...	276	291·6	0·83
T . .	138	118·0	...	138	115·9	4·21
OM .	132	117·2	...	132	138·3	0·29
OMc .	5	7·7	...	5	4·5	0·06
OC . .	63	60·8	...	63	60·8	0·08
OCc . .	163	135·5	...	163	135·5	5·58
OCo .	125	115·2	...	125	115·2	0·83
OT . .	54	48·2	...	54	48·5	0·62
MC, McC .	23	31·4	...	23	35·6	4·46
MCc .	6	65·7	...	...	...	...
MCo, McCo	73	60·1	...	73	67·7	0·41
MT, McT	2	25·8	...	...	...	...
McCc .	4	5·6	...	4	2·4	1·07
CCo . .	30	28·6	...	30	28·6	0·07
CT . .	11	11·9	...	11	12·2	0·12
CcCo .	63	64·3	...	63	64·3	0·03
CcT .	24	26·5	...	24	26·9	0·31
CoT .	24	23·1	...	24	23·0	0·04
Totals .	3492	3492·0	...	3492	3492·4	23·96
Gene frequencies						
+ . .		0·4397			0·4279	
O . .		0·1865			0·1865	
M . .		0·0906			0·1061	
Mc . .		0·0073			0·0034	
C . .		0·0467			0·0466	
Cc . .		0·1039			0·1039	
Co . .		0·0884			0·0884	
T . .		0·0369			0·0372	
Totals .		1·0000			1·0000	

all fish fall in one of the groups, *MC, McC*; *MCo, McCo*; and *MT, McT*. Let *v* and *w* be the frequencies of *M* and *Mc* respectively, and *v'* and *w'* be the frequencies these genes would have if the fish in the combinations just mentioned were neither *M* nor *Mc*. *v'* cannot be determined by the formula but *w'* and $v'+w'$ can. Let d'_1 and d'_2 be

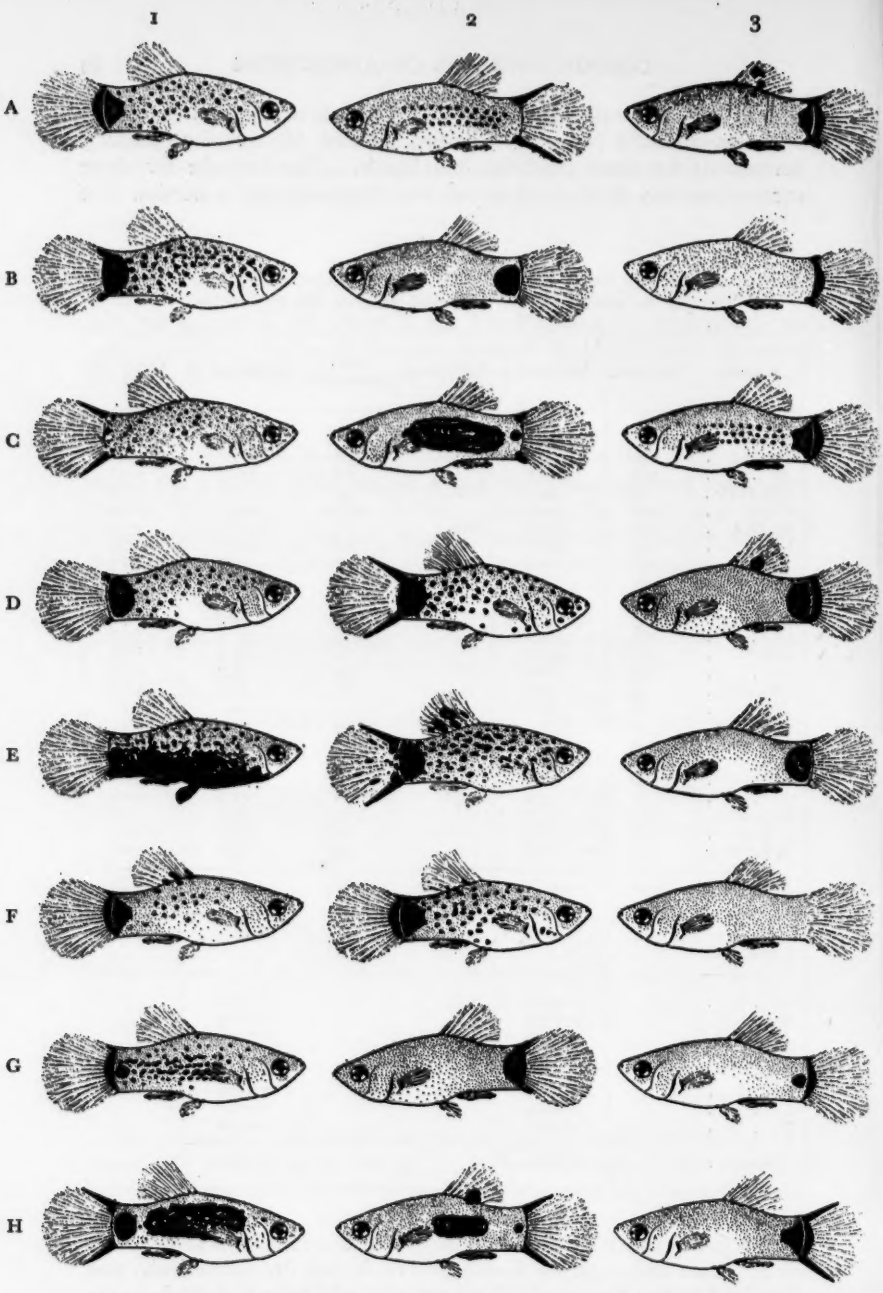

FIG. 1.—A random assortment of platyfish (*Platypoecilus maculatus*) from Mexico and Guatemala showing the great polymorphism in colour patterns. The tail patterns are referable to autosomal genes which form a multiple allelic series. The heavily spotted patterns on the body proper and on the dorsal fin are referable to sex-linked genes which form another multiple allelic series. (For identification of colour genes, see Key to Figures on opposite page.)

the portion of the fish in these combinations which are genotypically *M* and *Mc* respectively, then :—

$$d = d'_1 + d'_2$$

$$\frac{d'_1}{d'_2} = \frac{v}{w} \quad \text{(under theoretical conditions)}$$

Let :

$$d_1 = v - v'$$

$$d_2 = w - w'$$

Since only one of the two alleles of each of the fish in the combinations being considered is either *M* or *Mc* :—

$$2d_1 = d'_1$$

$$2d_2 = d'_2$$

$$d'_1 + d'_2 = d = 2(d_1 + d_2)$$

$$\frac{d_1}{d_2} = \frac{v}{w}$$

$$\frac{d_1 + d_2}{d_2} = \frac{v + w}{w}$$

$$\frac{d}{2d_2} = \frac{v + w}{d_2 + w'}$$

$$dd_2 + dw' = 2d_2v + 2d_2w$$

Key to Figures on opposite page.

Position	Autosomal alleles								Sex-linked alleles				
	+	*O*	*M*	*Mc*	*C*	*Cc*	*Co*	*T*	*Sp*	*Sr*	*Sd*	*Sb*	*N*
1. A	+	*O*	+	+	*C*	*Cc*	+	+	*Sp*	+	+	+	+
B	+	+	+	*Mc*	+	+	+	+	*Sp*	+	+	+	+
C	+	+	+	+	+	+	*Co*	+	*Sp*	+	+	+	+
D	+	*O*	+	*Mc*	+	+	+	+	*Sp*	+	+	+	+
E	+	+	+	+	+	+	+	+	+	+	+	*Sb*	+
F	+	+	+	+	+	*Cc*	+	+	*Sp*	+	*Sd*	+	+
G	+	*O*	+	+	*C*	+	+	+	*Sp*	*Sr*	+	+	+
H	+	+	+	*Mc*	+	+	*Co*	+	+	+	+	+	*N*
2. A	+	+	+	+	+	+	*Co*	*T*	+	*Sr*	+	+	+
B	+	+	*M*	+	+	+	+	+	+	+	+	+	+
C	+	*O*	+	+	+	+	+	*T*	+	+	+	+	*N*
D	+	+	*M*	+	+	+	*Co*	+	*Sp*	+	+	+	+
E	+	+	+	*Mc*	+	+	*Co*	+	*Sp*	+	*Sd*	+	+
F	+	+	+	+	+	*Cc*	+	+	*Sp*	+	+	+	+
G	+	*O*	+	*Mc*	+	+	+	+	+	+	+	+	+
H	+	*O*	+	+	+	+	*Co*	+	+	+	*Sd*	+	*N*
3. A	+	+	+	+	+	*Cc*	+	*T*	+	+	*Sd*	+	+
B	+	+	+	+	*C*	+	+	*T*	+	+	+	+	+
C	+	*O*	+	+	+	*Cc*	+	+	+	*Sr*	+	+	+
D	+	+	*M*	+	*C*	+	+	+	+	+	*Sd*	+	+
E	+	*O*	*M*	+	+	+	+	+	+	+	+	+	+
F	+	+	+	+	+	+	+	+	+	+	+	+	+
G	+	*O*	+	+	*C*	+	+	+	+	+	+	+	+
H	+	+	+	+	+	*Cc*	*Co*	+	+	+	+	+	+

$$
\begin{aligned}
d_2[d-2(v+w)] &= -dw' \\
d_2 &= \frac{dw'}{2(v+w)-d} \\
d_2 &= \frac{dw'}{2\left(v+w-\frac{d}{2}\right)} \\
d_2 &= \frac{dw'}{2(v'+w')} \\
w &= w'+\frac{dw'}{2(v'+w')} \\
w &= w'\left[1+\frac{d}{2(v'+w')}\right] \quad . \quad . \quad (1) \\
v &= (v+w)-w \\
v &= v'+w'+\frac{d}{2}-w \quad . \quad . \quad . \quad (2)
\end{aligned}
$$

(1) and (2) were used to find first w and then v.

For both the Rio Jamapa and the Rio Papaloapan populations the frequency of + was found by subtracting the total of all the other gene frequencies from 1.

In the second calculation of the gene frequencies in the Rio Papaloapan population, some of the phenotypes contained so many genotypes that a different procedure was used to find the gene frequencies. This new method is a generalisation of the use of the formula $a = 1-\sqrt{1-x}$; this accounts for the values of the frequencies of several genes being the same in both calculations.

Each genotype should occur in the proportion a^2 if it is homozygous or $2ab$ if heterozygous, a and b being the gene frequencies involved. Since :—

$$
\begin{aligned}
(a_1+a_2+\ldots+a_n)^2 = {} & a_1^2+a_2^2\ldots+a_n^2 \\
& +2a_1a_2+2a_1a_3+\ldots+2a_1a_n \\
& +2a_2a_3+2a_2a_4+\ldots+2a_2a_n \\
& \quad + \\
& \quad \vdots \\
& +2a_{n-1}a_n
\end{aligned}
$$

$$
i.e. \left(\sum_{i=1}^{n} a_i\right)^2 = \sum_{i=1}^{n}\sum_{j=1}^{n} a_ia_j
$$

the squares of the sums of certain gene frequencies can be found by adding together the proportion observed for each of certain genotypes. The sum of these gene frequencies is found by taking the square root. Because, in some cases, more than one genotype has the same phenotype, not all such sums of gene frequencies can be found. By correctly choosing eight (the number of genes in the allelic series) sums,

equations which can be easily solved for the gene frequencies may be obtained. The eight equations should be chosen so that each contains as many gene frequencies as possible and the equations have one and only one solution.

Let the following symbols represent the gene frequencies of the eight allelic genes :—

Frequency of $+$ $= p$
" O $= q$
" M $= v$
" Mc $= w$
" C $= t$
" Cc $= u$
" Co $= s$
" T $= r$

The equations used are :—

$$p+q+v+w+t+u+s+r = 1{\cdot}0000 \quad . \quad . \quad . \quad (1)$$
$$p \quad +v+w+t+u+s+r = 0{\cdot}8135 \quad . \quad . \quad . \quad (2)$$
$$p+q+v+w+t \quad +s+r = 0{\cdot}8961 \quad . \quad . \quad . \quad (3)$$
$$p+q+v+w+t+u \quad +r = 0{\cdot}9116 \quad . \quad . \quad . \quad (4)$$
$$p+q+v+w \quad +s+r = 0{\cdot}8494 \quad . \quad . \quad . \quad (5)$$
$$p+q \quad +t \quad +s+r = 0{\cdot}7866 \quad . \quad . \quad . \quad (6)$$
$$p \quad +t \quad +s \quad = 0{\cdot}5629 \quad . \quad . \quad . \quad (7)$$

In each of these seven equations either both v and w or neither v nor w appears. The eighth equation must give the value of $p+q+v$ or $p+v$, the only such sums which can be obtained and which contain only one of the two frequencies, v and w. There is a deviation of the observed number for O from one that can be calculated on the basis of the gene frequencies of $+$ and O which can be obtained without determining the frequencies of M and Mc. In addition, in finding $p+v$ only two observed values are used. Therefore, another method was used to determine v and w.

The gene frequencies were calculated from the seven equations with the sum of the frequencies of M and Mc being calculated instead of these frequencies. Then values of each phenotype were calculated using these gene frequencies. Genotypes were grouped together as though M and Mc were alike phenotypically so that the sum of their gene frequencies was sufficient for these calculations. The biggest difference between the calculated and observed values where M and Mc are involved was found to occur in the phenotypes M, Mc, MT, McT, MMc. The difference in Cc, CCc, MCc is ignored because the M fish form only a small part of this group. In order to make the goodness-of-fit of the observed values to the calculated numbers as nearly perfect as possible, the difference between them in the case of M, Mc, MT, McT, MMc was divided between M and Mc, MT, McT, MMc in proportion to the observed values for these groups of patterns. This made sure that the total contribution to χ^2 from these

groups was minimal.* Dividing the difference in this way, the calculated value of the M phenotype is obtained. From this value, V, and the frequency of $+$, p, the frequency of M, v, can be obtained because :—

$$2pv+v^2 = V$$
$$v = -p+\sqrt{V+p^2}$$

Then the frequency of M can be subtracted from the sum of the frequencies of M and Mc to find the frequency of Mc.

In each of the two calculations from the Rio Papaloapan population and the one for the Rio Jamapa, after the gene frequencies were found, the number of fish of each pattern which were expected on the basis of these gene frequencies was found where this had not already been done. Except for the first calculation of the Rio Papaloapan population, the goodness-of-fit of the observed values of these expected values was then found by means of the χ^2 test.†

ANALYSIS

If the gene frequencies are obtained for the Rio Papaloapan sample and the number of fish that would be expected to show each pattern on the basis of these gene frequencies is calculated, certain discrepancies between these calculated and the observed numbers

* If C is the total contribution to χ^2, e_1 and e_2 the calculated values which are to be found, k their known sum, and o_1 and o_2 the respective observed values :—

$$C = \frac{(e_1-o_1)^2}{e_1}+\frac{(e_2-o_2)^2}{e_2}$$
$$e_1+e_2 = k$$
$$C = \frac{(e_1-o_1)^2}{e_1}+\frac{(k-e_1-o_2)^2}{k-e_1}$$
$$C = e_1-2o_1+\frac{o_1^2}{e_1}+k-e_1-2o_2+\frac{o_2^2}{k-e_1}$$
$$\frac{dC}{de_1} = 1-\frac{o_1^2}{e_1^2}-1+\frac{o_2^2}{(k-e_1)^2} = 0$$
$$\frac{o_2^2}{(k-e_1)^2} = \frac{o_1^2}{e_1^2}$$
$$\frac{o_2}{e_2} = \pm\frac{o_1}{e_1}.$$

It is obvious that when the sign is $+$, this condition gives C its minimum value with positive e_1 and e_2.

† Dr Sewall Wright, in a letter, suggested that the gene frequencies for the Rio Papaloapan population be calculated by a different method. This method involved grouping *McCc* with *Cc*, *CCc* and *MCc*. By this method the frequencies of *O*, *Cc* and *Co* were calculated by the formula $a = 1-\sqrt{1-x}$. The frequency of *C* in platyfish without *Cc* (*Cc* conceals the presence of *C*) was found by the formula and multiplied by the total frequency of genes other than *Cc* to find the frequency of *C* in the entire population. In the same way, the frequency of *T* and the sum of the frequencies of *M* and *Mc* were determined. The frequency of + was found by subtracting the total of the other frequencies from 1. The total frequency of *M* and *Mc* was divided between *M* and *Mc* in proportion to the observed frequencies of *OM* and *OMc* respectively. The results of this method were, as Dr Wright calculated, approximately the same as those obtained by the method described above.

occur. The most striking of these are between the numbers for the phenotypes *MCc* and *MT*, *McT*. The calculations indicate that more +, *O*, *Co*, *MC* and *McC*, *MCc*, *McT* and *MT* fish should have been observed. They also show that too many *M*, *MMc* and *Mc*, *CCc* and *Cc*, *T*, *OM*, *OC*, *OCo*, *McCo* and *MCo* fish were observed as compared to the calculated values. The observed values for *C*, *OMc*, *OC*, *OT*, *McCc*, *CCo*, *CT*, *CcCo*, *CcT* and *CoT* are about correct. Here, as in the following similar statements, the deviations are listed without regard to significance ; most of the deviations can be considered to be caused by chance. An examination of the appearance (see fig. 1) of the patterns *MCc* and *MT*, *McT* reveals a strong probability that, if observed, most *MCc* fish would be considered to be *Cc* alone and most of the *MT* and *McT* fish would be taken for *Mc* alone.

Assuming that this was what occurred, some of the discrepancies can be corrected and others are lessened. The value of χ^2 is now 23·96 ; the corresponding probability is about 0·07. The observed frequencies of *O*, *Cc* and *CCc*, *MCc*, *Co*, *MC* and *McC* are too small. Those of *M*, *C*, *T*, *OCc* and *OCo* are too large. The observed numbers for +, *Mc*, *MMc*, *McT* and *MT*, *OM*, *OMc*, *OC*, *OT*, *McCo* and *MCo*, *McCc*, *CCo*, *CT*, *CcCo*, *CcT* and *CoT* are about equal to the calculated numbers.

In the Rio Jamapa population the situation is simplified by the absence of *M* and *Mc*. The value of χ^2 is 42·84. The probability is less than 0·001. The deviation between expected and observed values for *O* is similar to that in the Rio Papaloapan ; the other deviations in the Rio Jamapa are dissimilar to the corresponding Rio Papaloapan deviations. The observed values larger than their expected values are *CCc*, *Cc*, *T* and *OCo*. The observed values smaller than their expected values are *O*, *CcCo*, *CcT*, *CoT*. The nearly equal values occur for +, *C*, *Co*, *OC*, *OCc*, *OT*, *CCo*, *CT*.

DISCUSSION

The calculation for Gordon's (1947) observed values of *MCc* and *MT*, *McT* in the Rio Papaloapan population of platyfish give such a poor fit as to require some correction. If the gene frequency of *M* was assumed to be low enough for a reasonable fit for *MCc*, then the calculated values for the other patterns containing *M* would be so greatly reduced as to make the fit of observed to calculated values extremely bad for these patterns. Therefore, unless a different method of inheritance from that assumed in the calculations is involved, the fish cannot all actually have the patterns they appear to have.

A critical examination of the appearance of the patterns *MCc* and *MT*, *McT*, reveals that *MCc* would almost be the same as *Cc* alone and *MT* and *McT* would appear like *Mc*. Combining the *MCc* fish with the *Cc* fish, *i.e.* not attempting to separate *MCc* fish

from *Cc* fish in either the observations or calculations, and the *MT* and *McT* with *Mc* fish, allows new gene frequencies to be calculated. The new expected values and the regrouped observed values have a goodness-of-fit which is satisfactory. This goodness-of-fit indicates that the hypothesis that *MCc* fish were counted as *Cc* and that *MT* and *McT* fish were counted as *Mc* is satisfactory with reference to the consistency of the regrouped observed values with each other as well as the appearance of the patterns. It is therefore likely to be correct.

There are other possibilities. If random values totaling one are chosen for the gene frequencies, and expected values are calculated on the basis of these gene frequencies, some of the patterns will have been observed too frequently and others too infrequently as compared with these calculated values. If it is assumed that some of the fish in the groups with too high observed values actually should be in the groups with too low values, a satisfactory fit can be obtained. Two points must be kept in mind, however. First, except in the case noted above, patterns which seem to be phenotypically distinguishable from each other would have to be considered to be indistinguishable at least some of the time. Second, except in a few cases, the number of patterns which would have to be assumed indistinguishable is large.

There is the possibility that the *MCc* fish were counted as *M*, or that some were counted as *M* and others as *Cc*, and, as was assumed above, the *McT* and *MT* fish as *Mc*. However, the combination *MCc* appears like *Cc* rather than *M*.

In the Rio Jamapa the deviations of observed from calculated values are clearly significant. No simple explanation of the deviations is apparent. Any explanation must take into account that deviations similar to those in the Rio Jamapa sample do not occur in the Rio Papaloapan sample. Since this is so, the appearance of the single patterns and combinations, which are the same in both rivers, cannot be used to explain the situation in the Rio Jamapa sample as they were used for the Rio Papaloapan sample. (The particular patterns so used for the Rio Papaloapan calculations : *MCc*, *McT* and *MT*, do not occur in the Rio Jamapa population.) If it is assumed that incorrectly calculating the gene frequencies causes the deviations in the cases of *CcCo* and *CcT*, correcting the gene frequencies would produce even more significant deviations for at least some of the other patterns containing *Cc*, *Co* or *T*.

The combinations of *CcCo* and *CcT* cannot be less viable than the other patterns, unless other patterns of *Cc*, *Co* or *T* are more viable to an extent which just maintains the gene frequency at equilibrium. The fish which should have *CcCo* and *CcT* may exist but have patterns other than *CcCo* and *CcT*. This shift from one pattern to another would then be assumed to be produced by something which exists in the Rio Papaloapan and not in the Rio Jamapa. This factor might be a difference in the viability of the patterns which causes

deficiency of *CcCo* and *CcT* fish while producing an excess of other patterns containing *Cc*, *Co* or *T*. Another possibility is that this factor changes the patterns of genotypically *CcCo* and *CcT* fish so that these patterns resemble other patterns. Still other patterns may also be affected by this factor.

In commenting on the manuscript of this paper, Dr Wright said : " The mechanism by which so many alleles are kept in circulation is the most interesting question here. The simplest is, of course, a selective advantage of heterozygotes over both homozygotes. There doesn't seem to be much evidence for this but the amount of advantage needed is so slight that it might be difficult to detect. It can only be done in any case where there is no overlap of classes. Another possible mechanism is a selective advantage of any gene when rare as compared with when common."

Dr Ernst Mayr, in a letter, pointed out that in the case of *O* in the Rio Jamapa sample the number of fish with *O* alone was too small compared with the number with *O* in combination with another pattern. Since all fish homozygous for *O* are part of the former group, he stated that this discrepancy, although not significant, suggested a selective advantage of heterozygotes over homozygotes. However, other discrepancies occur which can not be explained in this way, *e.g.* the excess of single *C* and *T* in the Rio Jamapa sample. In this sample, 511 fish with single patterns were observed while only 504·9 were expected. Thus no selective advantage of heterozygotes over homozygotes is indicated by the data. It must be pointed out, however, that these data, owing to pattern complexities, are not particularly suitable for the determination of selective advantages of the various genotypes.

CONCLUSIONS

Using the data of Gordon (1947) on phenotypic frequencies of populations of the polymorphic Mexican platyfish (*Platypoecilus maculatus*) from the Rio Jamapa and Rio Papaloapan, the gene frequencies were calculated by several formulæ derived from Hardy's Formula. These gene frequencies were then used to calculate the ratios of all the phenotypes that would be expected from them and the goodness-of-fit, of the observed phenotypic ratios to the calculated ones, determined by the χ^2 test.

The seven autosomal, dominant, allelomorphic genes for tail patterns found in the Rio Papaloapan platyfish can be recognised when occurring singly, but some of their combinations are indistinguishable. That the patterns *MCc*, *McT* and *MT* were not observed in sufficient numbers is indicated. Fish with these patterns were probably considered to have the pattern *Cc* when they had *MCc*, and *Mc* when they had *McT* and *MT*. If the observed numbers of fish are regrouped on the supposition that *MCc* fish cannot be distinguished phenotypically from *Cc* fish in most cases, nor *McT* and *MT* fish from *Mc* ones, the

various combinations and single patterns are found to occur in frequencies consistent with each other.

In the Rio Jamapa population the complicating patterns *M* and *Mc* are missing. This simplified its analysis somewhat. However, the dual *CCc* pattern which occurs in the Rio Jamapa population is indistinguishable from the single *Cc* pattern. Allowing for this difficulty, discrepancies still exist among the observed frequencies of single patterns and the various combinations of those patterns. An explanation for this is lacking at the present time.

The Rio Papaloapan and Rio Jamapa populations differ with respect to whether the tail patterns *CcCo* and *CcT* occur with a frequency consistent with that predicated from the gene frequencies of *Cc*, *Co* and *T*.

In both populations the frequency of single patterns, as employed by Gordon (1947), is an adequate indication of the gene frequency of the gene responsible for that pattern.

SUMMARY

Seven autosomal, dominant multiple alleles produce a series of seven pigment patterns in the tail region of *Platypoecilus maculatus*, the common aquarium platyfish. In most cases, but not in all, different genotypes for tail patterns may be phenotypically distinguished. The observed frequencies of the various patterns were compared with each other by calculating the frequencies of the corresponding genes. In the Rio Papaloapan population the frequencies of the patterns are consistent with each other if certain similar appearing patterns are grouped and evaluated together. In the Rio Jamapa population, on the other hand, the frequencies are not consistent with each other.

In the two platyfish populations analysed, from the Rio Jamapa and the Rio Papaloapan (and presumably also from two other rivers, the Rio Coatzacoalcos and the Rio Usumacinta) the determination of the frequency of an individual pattern, uncombined with other patterns, is an adequate indication of its corresponding gene frequency.

The authors are indebted to Dr Howard Levene, Columbia University, for the following comment: " It should be noted that the fit would have been somewhat better, and χ^2 would have been reduced, if the gene frequencies had been estimated by the method of maximum likelihood. However, the χ^2 for the Rio Jamapa population would almost certainly still be significant. Since the conclusions reached would be essentially unaffected, and the uncertainties of classification make extreme precision impossible, the considerable labor of computing maximum likelihood estimates does not seem necessary."

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THE SUPPLY OF RIBONUCLEIC ACID TO THE MALE GERM CELLS DURING MEIOSIS IN *ASELLUS AQUATICUS*

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1. INTRODUCTION

In the course of a study on the spermatogenesis of the isopod *Asellus aquaticus* L. (G. Montalenti and G. Vitagliano, 1946; G. Vitagliano, 1947) attention was attracted to a layer of big secretory cells surrounding each testicular lobe or follicle. The large nuclei of these follicular cells are polyploid, similar to those described by Geitler (1939) in *Gerris*. Considerable variation in their structure was noticed. The variations were supposed to be correlated with endomitotic cycles. This hypothesis, however, had to be discarded because, if true, endomitosis would have been too frequent and the nuclei should have increased their volume indefinitely. This was not the case and a connection between the structure of nuclei and phase of physiological activity of the cells seemed more likely.

The older cytologists had long argued about the participation of nucleus in the secretory phenomena of the cell and it was realised that *Asellus* might be very favourable for a study of this problem. In fact, one of the most difficult questions, namely the correct seriation of the stages, could be easily cleared up if the secretion process bore some constant relation to the course of spermatogenesis. The latter, being almost synchronous in all the elements of each lobe, provides a time scale to which the phase of secretory activity of the follicular cells can be referred.

A remarkable correlation between the structural aspect of follicular nuclei and the stage of spermatogenesis was found. At the same time it was realised that the secretion of the follicular cells is absorbed by the germ cells. Thus the problem shifted from secretion physiology towards the field of germ cell and meiosis physiology. The secretion was recognised as ribonucleic acid (RNA), which is presumably utilised in thymonucleic acid (DNA) synthesis during meiotic processes.

Preliminary reports of the essential results presented in this paper have already been published (G. Vitagliano, 1948 ; M. de Nicola, 1948 ; G. Vitagliano and M. de Nicola, 1948 ; G. Montalenti, 1948). The original problem, *i.e.* the participation of the nucleus in secretory processes, will not be dealt with in full in the present paper.

2. MATERIAL AND TECHNIQUE

Testes of *Asellus aquaticus* L. (from the river Sarno, near Naples) were examined by the squash method or in sections. Squash preparations were made by the acetocarmine (Schneider) or aceto-orcein (La Cour) methods, and occasionally restained by the Feulgen reaction, iron-hæmatoxylin or gentian violet. Material to be sectioned was fixed in Carnoy (acetic alcohol 1 : 3), Helly, Navashin, Flemming. Sections were stained with hæmatoxylin-eosin, iron-hæmatoxylin, Feulgen. The demonstration of ribonucleic acid was obtained by Unna-Pappenheim's stain with control by ribonuclease treatment, according to Brachet (1942). The demonstration of phosphatases was carried out by Gomori's technique (for technical details, *cf.* de Nicola, 1948).

Control observations were made in living material with normal and polarised light.

3. OBSERVATIONS

(*a*) *The structure of the* Asellus *testis*

In the mid-dorsal region of the thorax of the male *Asellus* a pair of long slender tubes, the ducti deferentes, run towards their openings located in the ventral part of the seventh thoracic segment. Towards the opposite apical end three pear-like lobes or follicles are appended to each ductus deferens. In each of the six testicular lobes spermatogenesis takes place independently, but within each lobe the course of the germ cells evolution is fairly synchronous. In every follicle three main kinds of cells can be distinguished :—

(1) An investing layer of big follicular cells showing secretory activity and similar, although not identical, with the cells constituting the " neck " of the follicle and the epithelium of the deferens.

(2) The group of evolving spermatocytes (or spermatids, or fully-formed spermatozoa) which occupies the largest part of the follicular cavity.

(3) A small group of spermatogonia, located somewhere towards the basal part of the follicle ; these are the source of the next spermatogenetic wave. When all the spermatocytes have been converted into spermatozoa, they are poured through the neck of the follicle into the ductus deferens.

(*b*) *The concordance between the rhythm of activity of the follicular cells and the stages of spermatogenesis*

As a preliminary observation it was found that two main " phases " exist in polyploid nuclei, *viz.* one characterised by large nuclear size, chromatin distributed in small blocks spaced within a non-staining nuclear sap, and the other characterised by smaller nuclear size and chromatin condensed in a mass in which the single blocks are not so easy to recognise. The former aspect is very similar to *Gerris* nuclei as described by Geitler (1939). The individual blocks may represent single chromosomes. No attempt was made to count them in order to ascertain the degree of ploidy, but apparently this is not very high, *e.g.* of the order of 8 to 16 ($2n = 16$ in *Asellus*).

By a more careful examination four " phases " were established and their correspondence with spermatogenetic stages thoroughly worked out by the study of some fifty sectioned testicular lobes and of a great number of squash preparations. The results of the observations are presented in the following table and in figs. 1 and 2.

A few words may be added to explain some points. In a testicular lobe full of spermatozoa the great majority of the follicular cells are in phase I. But in proximity to the group of spermatogonia, which are ready to start the next spermatogenetic wave as soon as the spermatozoa have been expelled, the follicular cells are in phase II.

Possibly two stages are included in phase II, the first with clearly visible threads, the second in which the aspect of the nucleus is more uniform. The interpretation is that the chromosomes (= chromatin blocks of phase I) are now threadlike, *i.e.* much less contracted and less charged with DNA. The whole chromatin structure is thus in a more diffuse condition.

Some additional nucleoli appear in phase II. One or two (which we consider identical with those of the other stages) are pyronin-positive and contain both kinds of phosphatases. Three to six additional nucleoli are devoid of RNA and of diphosphatases.

Phase III corresponds apparently to the stage of more intense secretion.

Phase IV is interpreted as a transitional stage to phase I.

The correspondence between spermatogenesis and follicle cell phase is such that in each spermatogenetic cycle phase I and IV appear only once, as the starting and final stage respectively, while phase II and III appear twice. There are thus two secretory waves with the maxima corresponding, one to lepto-zygotene, and the other to diakinesis-anaphase I.

The secretion is constituted essentially by RNA (perhaps it is pure RNA) which, according to our observation, is absorbed and in some way utilised by the germ cells.

At the end of spermiohistogenesis no RNA is found between spermatozoa while the follicular cells have reached phase I, in which no secretory activity can be detected.

TABLE 1

Follicular cells

Phase	Nucleus	Nucleoli	Cytoplasm
I	Large. Chromatin in small blocks of irregular shape. No big chromocenters. Mp and Dp+ on chromatin blocks.	One or two, faintly stained by pyronin.*	Uniform, devoid of secretion granules. Very faintly stained by pyronin.
II	Larger than in phase I. Chromatin in diffused condition. With aceto-carmine and iron-hæmatoxylin, a thread-like structure appears underneath the uniform diffuse staining. Mp reaction diffuse and slight. Dp −.	Four to seven (with iron - hæmatoxylin). Only one or two are stained by pyronin. Mp+ in all nucleoli. Dp+ in 1-2 nucleoli, in the others −.	Vacuolar and full of small granules, particularly abundant around the nuclear membrane. The granules stain intensely with pyronin. Mp and Dp −.
III	Much contracted. No granular structure visible. Granules, if existing, closely packed in a single mass. Mp and Dp ++.	One or two, intensely stained by pyronin. Mp and Dp ++.	Filled with big granules intensely stained by pyronin. They are expelled from the cell and flow towards the germ cells. Mp and Dp both absent from the granules.
IV	Large. Chromatin in blocks, less condensed than in phase I. Mp and Dp + on the chromatin blocks.	One or two, faintly stained with pyronin. Mp and Dp +.	Alveolar containing a few pyronin - positive granules. Mp and Dp −.

* The staining by pyronin is given as a proof of the presence of RNA, since control with ribonuclease was done in every case. For the sake of brevity this is not stated every time. Concerning criticism on the specificity of the reaction, see Brachet and Shaver, 1948.

As to the distribution of phosphatases in the germ cells, the following can be noted. The cytoplasm and extracellular RNA granules are always negative. Spermatogonial nuclei are always strongly positive both for mono- and diphosphatases. The same is true of the spermatocytic nuclei, where it is easy to see that phosphatases closely follow the distribution of chromatin. In young spermatocytes these enzymes are localised in the tail region, as is the chromatin. The whole head of the spermatozoa is positive.

(c) *Observations* in vivo

The diffused or condensed state of the chromatin can be detected also *in vivo*, although the details are of course much less clear. In polarised light the chromatin blocks of phases I and IV distinctly show birefringence, while in " diffuse " (phase III) nuclei no birefringence (*cf.* figs. 1 and 2).

Germ cells

Stage	Intracellular space	Nucleus	Cytoplasm
Late spermatids or fully formed spermatozoa.	No granules of RNA.	The distribution of phosphatases closely follows the distribution of chromatin.	...
Spermatogonia and pachytene.	Minute granules of RNA are distributed among the spermatogonia which are ready to initiate the next spermatogenetic wave. Practically no granules between spermatocytes in pachytene. Granules always devoid of phosphatases.	The distribution of phosphatases closely follows the distribution of chromatin.	Staining pink with pyronin in spermatogonia. Negative to pyronin in pachytene spermatids. Mp and Dp —.
Lepto - zygotene and diakinesis to anaphase I.	Large amount of RNA granules between the spermatocytes.	The distribution of phosphatases closely follows the distribution of chromatin.	Strongly positive to pyronin. Mp and Dp —.
Ana-telophase II or young spermatids.	RNA granules gradually disappear. Groups of young spermatids are often oriented with their tails towards dumps of granules.	The distribution of phosphatases closely follows the distribution of chromatin.	Positivity to pyronin gradually disappearing. Mp and Dp —.

++ means strongly positive Gomori reaction
\+ positive
— negative

Mp, monophosphatase
Dp, diphosphatase

is observed. The same is true for the germ cells ; when the chromatin is condensed (pachytene, metaphase, spermatozoa) a slight or strong (spermatozoa) birefringence is seen, which is lacking when the chromatin is in a " diffuse " condition (resting nuclei, prophase to pachytene).

(d) Ductus deferens

The epithelium of the *ductus deferens* is made up by elements similar to the follicular cells, although with a lower degree of polyploidy.

Phases analogous to those found in follicular cells are evident also in the deferens cells, but they are more or less intermingled. Their secretion has not been carefully examined, but it is probably constituted by large granules staining red with pyronin, which are not deleted by ribonuclease. This substance, which also stains deeply with iron-hæmatoxylin, obviously is not RNA, and is found in large amounts between ripe spermatozoa.

4. INTERPRETATION

(*a*) *The RNA cycle*

From the observation cited above it follows that the function of follicular cells is to secrete considerable amounts of RNA which are expelled in the form of granules and supplied to the germ cells. These in turn absorb it as is demonstrated by the stainability with pyronin of the cytoplasm of spermatogonia and spermatocytes at definite stages. The feeding of RNA shows two maxima during each spermatogenetic cycle : *viz.* at the beginning of meiotic prophase and at diakinesis-metaphase I.

The results of the phosphatase reaction help us to ascertain where a metabolism of RNA occurs. Since it is highly probable that these enzymes are concerned with RNA metabolism (Brachet and Jeener, 1946 ; Jeener, 1947 ; Montalenti and de Nicola, 1948) where phosphatases are absent there is probably no chemical elaboration of RNA. This happens during the whole migration of this substance, *i.e.* up to the cytoplasm of germ cells. Only in two points, *viz.* the starting point in follicular nucleus and the end point in germ cell nucleus, are the phosphatases present and in considerable amount. In other cells (*e.g.* other glandular cells, oocytes, etc.) in which RNA is present in cytoplasm, the phosphatases are also present in it. But here, according to the current views, RNA is in some way utilised for protein synthesis and thus presumably it undergoes change. Therefore the absence of phosphatases in the cytoplasm of both follicular and germ cells may indicate that it is carried through these bodies without any chemical modification.

The presence of phosphatases in nuclei, on the contrary, might indicate that there it is being metabolised. In follicular nuclei it is probably elaborated from the chromatin, whereas in germ cell nucleus it is eventually depolymerised into nucleotides which are used then to build up chromatin, *i.e.* DNA, according to the current view.

The supply of RNA to germ cells is thus considered as essential for completion of meiosis.

(*b*) *The secretory process*

As already stated the question of the participation of the nucleus in the secretory activity of the glandular cells has been long debated. Many older cytologists considered it very likely that the nucleus takes

an active part in secretion. It lies outside the purpose of the present paper to review the literature concerning such a subject, but a few words may be added to interpret the phenomena described above.

In this particular case in which the cell secretes RNA it is clear that the nucleus is in some way concerned in the elaboration of this substance. This is shown by the considerable variations (1) of nuclear size, (2) of the state of condensation of chromatin and (3) of the number and size of nucleoli. All these features have already been described in a great variety of cells by many cytologists. Amongst recent papers Painter's (1945) work on the glandular cells of the bee and *Drosophila* is particularly exhaustive.

In the present case the phosphatase content of the nucleus throws some light on the process. The highest quantity of phosphatases is observed in phase III, in which other signs indicate that the secretory activity reaches its highest level. Furthermore the phosphatase reaction, as well as other staining methods, clearly show that in phase II there are at least two kinds of nucleoli. Their specific function is not known, but it seems highly probable that they are in some way concerned with some process in RNA elaboration.

As to whether eu- or heterochromatin is the source of RNA, our data do not allow of any conclusion. In mitotic chromosomes heterochromatin in *Asellus* does not form considerable blocks, but is interstitial in its distribution. We do not know whether the chromatin blocks which we consider as chromosomes in polyploid nuclei are entire chromosomes or merely heterochromatic bodies, as will perhaps be considered more probable.

At any rate the conclusion that RNA is elaborated in the nucleus at the expense or with the co-operation of the chromatin cannot be avoided.

5. GENERALISATION AND DISCUSSION

Were the case of *Asellus* isolated, its value would be purely episodic and no general conclusion could be drawn. But it seems more likely that such a fine mechanism of supply of nucleic acid to the male germ cells is at work also in other organisms. According to our interpretation it is essential for completion of meiosis.

A glance at the literature confirms this view. Brachet (1942) found progressive diminution of RNA content in the cytoplasm during spermatogenesis in the mouse, rabbit, grasshopper, and planarians. He did not describe a two-phase cycle as in *Asellus*, nor did he look for the source of RNA. Painter (1940) in the *Rhoeo* anthers finds the same diminution of RNA during the meiotic process. Moreover, he states that the tapetal cells contain a great amount of RNA in their cytoplasm. In the post-meiotic stage these cells cytolise and set free RNA, which accumulates in the pollen grain. The author states that " there is a similarity between microspore growth at the expense of tapetal cells and growth of oocytes in animals through the agency

of nurse cells. Large amounts of ribonucleic acid are stored in mature pollen grain and used in the extremely rapid synthesis of new materials which accompanies pollen tube growth." He refers to his work (Painter and Reindorp, 1939) on the nurse cells of the ovary of *Drosophila*.

Oogenesis is a much more complex phenomenon than spermatogenesis: meiosis has a peculiar course owing to the cytoplasmic growth and elaboration and storage of yolk. The oocyte nucleus seems also directly concerned in supplying nucleic acids to the cytoplasm (Painter and Taylor, 1940, 1942). Therefore this matter will not be dealt with here. But it seems possible that the tapetal cells in *Rhoeo* anthers give RNA to the microspore also in premeiotic and meiotic stages.

A "higher concentration in the cytoplasm of nucleic acid" in mother cells in comparison with their neighbours is found also by Darlington and La Cour (1946) in *Fritillaria*. Moreover, they describe temporary pre-prophase chromatin (DNA) dumps, not associated with chromosomes, which gradually shrink, contemporary with chromosome individuation, and eventually disappear at leptotene. The dumps are a source of DNA for the chromosomes which have to start meiosis. This case is, as far as is known, unique.

Many data from the older literature point to the presence in other animals of mechanisms similar to those in *Asellus* follicular cells. In almost any order of insects peculiar cells (Verson's cell, apical cell) have been described by several authors. Almost all of them agree in two points: *viz.* the cells elaborate a granular basophilic material (sometimes considered as chondriome) and this is fed to the germ cells. The latter are often seen in close connection with the apical cell, generally at an early (spermatogonial) stage and sometimes they send pseudopodium-like structures towards the apical cell. This has been interpreted as a "nurse cell" supplying "food" to the germ cells, and McClung (1938) even thought that in this way some impression or experience of the soma could reach the germ cells. A recent review of the literature on the apical cells in several insect orders is to be found in Carson (1945).

Whether the basophilic material excreted by the apical cells and absorbed by the germ cells is RNA remains, of course, to be demonstrated. It seems very probable and at least in one case, in grasshopper testis, it has been proved (B. Battaglia, in press).

Important observations were made by Holmgren (1901) in the testis of *Staphylinus*. First, the apical cell is not the only organ showing the secretory activity: the membrane of the single spermatogonial cysts also produces the same basophilic granules, and even the follicular capsule, syncytial in its structure, seems to have a high cytoplasmic basophily. Similar conditions have been found in grasshopper testes in "connective tissue cells." Probably this is the way by which a further amount of RNA is supplied to spermatocytes sometimes during

the course of meiosis, as is in *Asellus* effected by the second climax of secretion.

A second and more important observation by Holmgren was that the size and shape of the apical cell, as well as of the whole follicular capsule varies very much according to the season and spermatogenetic stage. In winter, while spermatogenesis is in progress, both apical cell and capsule are thick and apparently very active. In spring, when meiotic stages come to their end and spermiohistogenesis starts, the apical cell is much smaller. In summer, when the testis is full of spermatozoa and devoid of spermatogonia and spermatocytes, Holmgren does not find the apical cell, and the capsule is reduced to an extremely thin layer. The activity of these secretory organs is therefore clearly connected with spermatogenesis as in *Asellus*.

Also in a Pteropode (*Hyalocylis striata*) the follicular cells surrounding the male section of the hermaphroditic gonad show a cycle of secretion of RNA, which is absorbed by the germ cells (Vitagliano and Bacci, unpublished).

In the vertebrates probably the Sertoli cells, in which Brachet finds a high content of RNA, have the same function.

There is no doubt that, looking more carefully into the literature and studying directly organisms of different types, it will be found that the supply of RNA to the male germ cells is provided for in a somewhat similar manner, although by different kinds of cells or cellular complexes. Research is in progress along this line in our laboratory.

The question may now be raised as to what is the causal relationship of this high charge of RNA supplied to the germ cells and the start and completion of meiosis. Darlington and La Cour (1946) have already stated that " what sets the series of changes (which will lead to crossing over, segregation and the whole course of meiosis) in motion seems to be the premature priming of the chromosomes with nucleic acid (premature with respect to their reproduction)." Modern research has demonstrated that meiosis is by no means confined to germ cells. Somatic cells are able to undergo more or less complete meiotic processes. In plants a number of cases have been described. E. Battaglia (1946) and E. Battaglia and Dolcher (1947) have carefully described incomplete and complete meiotic processes occurring in some glandular cells located at the basal part of the stilar canals in *Sambucus*. They did not look for RNA content in the cytoplasm of these cells, but two facts indicate that it should be rather high. First, the basophily of the cytoplasm, which is expressly described by the authors and appears also from the microphotograph accompanying their paper. Secondly, by the fact that Painter in *Rhoeo* found a considerable charge of RNA in the pistil, along the stilar canal. By analogy it may be supposed, while waiting for a demonstration, that this is true also in *Sambucus*.

In animals some cases of chromosomes pairing have been found in

tumour cells, and particularly in those classified in category A by Santesson and Caspersson (1942), *i.e.* elements whose cytoplasm is heavily charged with RNA. Descriptions of such cases are to be found in Evans and Swezy (1929), Barigozzi and Cusmano (1946, 1947). Hearne (1936) and Hearne Creech (1940) found similar phenomena in mice fibroblasts cultivated *in vitro* and treated with carcinogenetic hydrocarbons.

A true " somatic reduction " has been described in polyploid cells of *Culex* (Berger, 1938 ; Grell, 1946). This process is very complicated and nothing is known about its determination or the RNA content of the cells concerned. Therefore this case cannot be taken into consideration for the moment.

From these data (and no doubt many more will be found in the literature) it appears that (*a*) RNA supply from the exterior to male germ cells immediately before and/or during meiosis should be a widespread feature among animals ; (*b*) somatic cells can undergo more or less complete meiotic processes ; a common feature in the latter seems to be a heavy charge of RNA in the cytoplasm.

Therefore it may be supposed that a large amount of RNA is the cause, or at least one of the causes of the starting of meiosis. Possibly some enzyme might be bound to RNA and directly concerned in control of some meiotic process. Only experiment, of course, can give an answer. Some experimental data, however, are already at hand. Huskins (1948*a*) shortly reports that Kodani, by treating onion roots with sodium ribonucleate, obtained " chromosome pairing and reduction divisions, similar in essential respects to those of the germ cells." Similar cases have also been observed in material in which mitosis was induced by indole 3 acetic treatment. Apparently with sodium ribonucleate the number of reduction divisions is greatly increased.

In subsequent papers Huskins (1948*b*) and Wilson and Cheng (1949) describe their findings in *Allium* and *Trillium* roots following the treatment with sodium ribonucleate. They have obtained segregation of homologous chromosomes, but pairing " has been found so far in very few nuclei " (Huskins, 1948*b*).

Further observational and experimental data are required before any definite conclusion can be arrived at, but from this preliminary account it appears that the above-stated hypothesis is worth consideration.

6. SUMMARY

1. In the testis of *Asellus aquaticus* large follicular cells with polyploid nuclei are present. They are secretory elements, whose rhythmical work is in phase with the course of meiosis.

2. Follicular cells secrete ribonucleic acid (RNA) in granular form. During one spermatogenetic cycle the intensity of secretion shows two peaks : the first corresponding to lepto-zygotene, the

second to diakinesis-metaphase I. Some features of the polyploid nuclei during the various phases of activity are shortly described.

3. The secretion given off from the follicular cells goes to the germ cells which absorb and utilise it as is demonstrated by the presence of RNA in their cytoplasm at certain stages, and its absence in other stages.

4. Phosphatase reaction shows that these enzymes are present in the follicular nuclei and germ cell nuclei, absent in the cytoplasm of both kinds of cells and on the RNA granules.

5. Therefore it is assumed that RNA is elaborated in polyploid nuclei and used in germ cell nuclei to build up chromatin (DNA).

6. Similar mechanisms of RNA supply to male germ cells seem quite widespread among animals. Thus the hypothesis is proposed and discussed that a heavy RNA charge, coming from outside the germ cell, is causally connected with the starting of meiosis.

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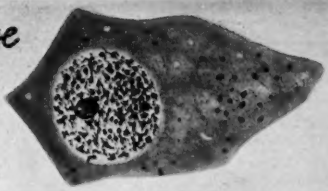
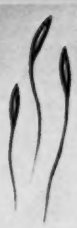
FIG. 1.—Diagram showing the concordance between the rhythm of activity of the follicular cells (on the left) and the stage of the spermatogenesis (on the right). Further explanation, see table 1.

Phase

I





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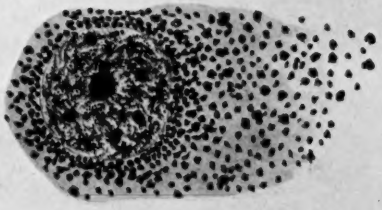

SPERMATOGONIA

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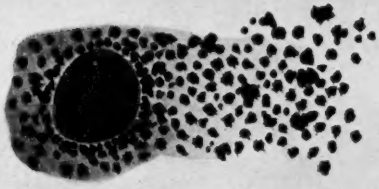
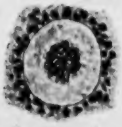
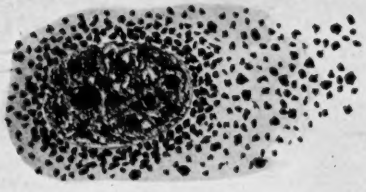




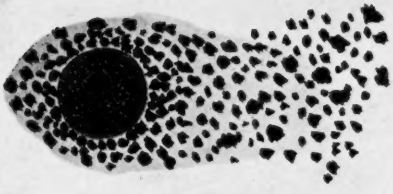
LEPTO-
ZYGOTENE

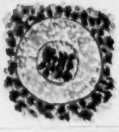
II



PACHYTENE

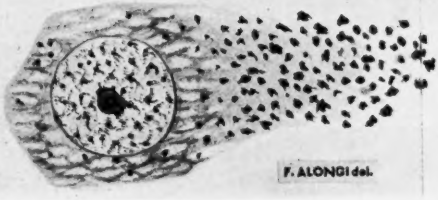
III





DIPLOTENE-
METAPHASE I

IV



F. ALONGI del.

SPERMATIDS

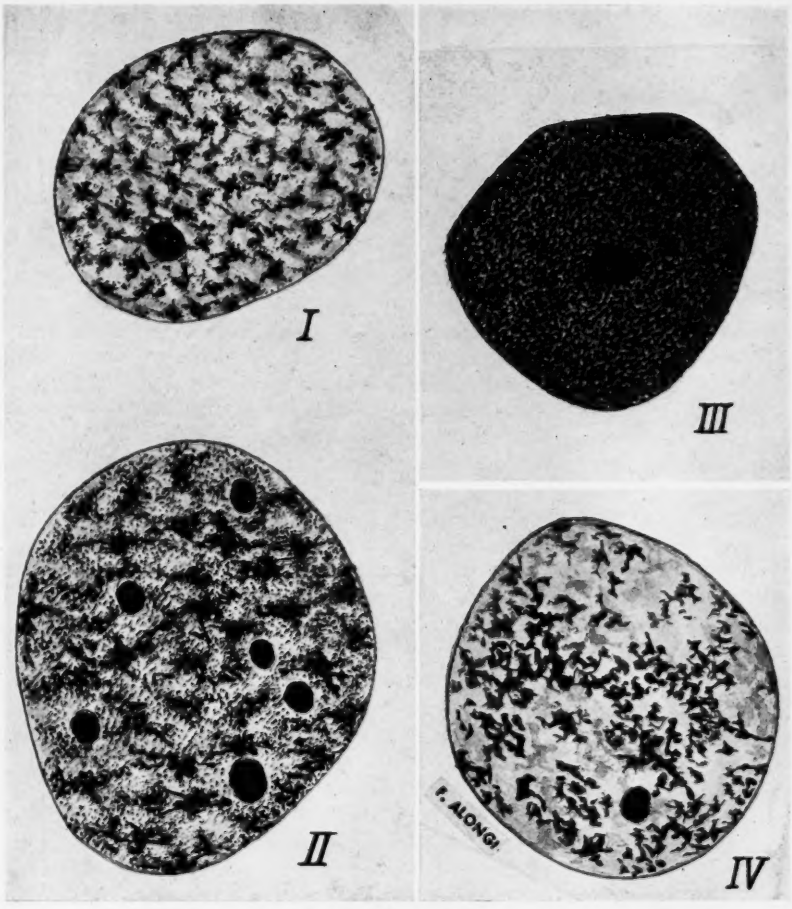


FIG. 2.—Nuclei of follicular cells corresponding to the phases indicated by the Roman figures below (*cf.* table 1).

THE DEVELOPMENT AND MAINTENANCE OF SUPERIOR GENETIC STOCKS *

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I. THE COST-BENEFIT RATIO

THE increase in the world's resources of primary products which scientific plant breeding has brought about in scarcely more than a man's span of life cannot even approximately be estimated. It has raised the output of one crop or another in every part of the globe. There are instances of spectacular yield increases which have affected the food habits and improved the living standards of more than one continent. Hybrid maize, now almost universal in the United States, has raised yields by 25 per cent. over the varieties previously grown, adding some 700 million bushels a year to the world's grain resources.[30] Improved varieties of wheat have increased the total wheat production in the U.S.A. by more than 800 million bushels in the years 1942-46.[3] In the prairie provinces of Western Canada the new stem-rust resistant wheats have produced an estimated annual yield increase of 41 million bushels valued at 27 million dollars.[4]

Modern varieties have raised the yield of winter wheat in Sweden by about 30 per cent. through combining high yielding capacity with winterhardiness, disease resistance and stiff straw.[1]

We must now consider the effort and expense involved in introducing techniques which have had such remarkable effects. Plant breeding is perhaps unique among resource techniques in requiring from the user little effort, in fact, as a rule, no change in management at all. The whole of the effort is confined to devising the new technique and this entails expenditure which, as a rule, is infinitesimal in comparison with the advances in productivity for which it is responsible. We have been instructed to place emphasis in our discussions on the " economic costs and benefits of the application of a given technique." We can say with conviction that the cost-benefit ratio which plant breeding can claim will be hard to rival in any other branch of agricultural science. Plant breeding establishments are modest places with regard both to personnel and equipment; and once an improved variety is obtained, it goes on earning dividends, at best—as in self-fertilising and asexually propagated plants—at the modest cost of purification and certification,

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at worst—as in hybrid maize—at a cost which is small in proportion to the benefit it helps to spread. One may venture the generalisation that, so far, there probably has not been one plant breeding scheme—provided it was sensibly conducted—that has failed to pay a dividend. This is true not only for projects serving vast areas. In New Zealand the yield increase brought about by a new variety, which is grown in an isolated pocket of less than 3000 acres of wheat, repays the annual cost of all wheat breeding in that country.

But the cost-benefit ratio is not merely an absolute statistic; there is a relative component—as between plant breeding schemes—which we must bear in mind particularly at this Conference. Yield increases may be obtained in a variety of ways,[10] in the small-grained cereals, for example, by increasing the number of heads per plant, or the number of grains per head, or the weight of a single grain, or by a combination of any of these; in short, by a direct improvement of what has been called the "yield characters." We may call this *production breeding*. On the other hand it is possible to achieve a similar effect, not by improving the "production genes" themselves, but by avoiding yield losses; by removing, or reducing, the effect of some factor which limits yield. One may call this *resistance breeding*. In practice the two can scarcely be separated; but they receive a different emphasis in every breeding scheme.

It is obvious that, from the viewpoint of utilising resources of soil fertility, manpower, machinery, etc., the second kind of approach is the more valuable. A crop which suffers damage will often require little less of soil resources and human effort than one which remains intact, the more so the later in the life cycle the damage occurs. A crop ruined in the seedling stage can often be replaced at relatively slight cost; but a mature crop which sheds its grain, or a plantation a generation old destroyed by parasites, is a more serious loss. "Production breeding" as a rule demands an additional investment, in particular in plant foods; "resistance breeding" insures the safety of an investment which has already been made. In the first case there is a recurrent debit item to the cost account, in the second there is not.

These observations illustrate a fact which, I believe, should be foremost in our minds when we consider plant breeding as a major factor in the utilisation of land resources: that there are policy considerations which transcend the temporary economic or national interests of the day. There are others. We must consider the claims of projects to improve long-lived crops, such as forest trees, where the benefit will accrue to future generations; or of long-range projects of distant and uncertain promise. Looking at the world at large, we must consider whether the scientific resources, at a time of acute shortage of trained and experienced workers, are best employed where they are or whether some of them should be re-deployed in countries where plant breeding as yet finds wide empty spaces. But

first we must pass in brief review the main problems of, and techniques for, breeding and maintaining high-yielding crop plants.

2. BUILDING STONES

The first stage in any breeding project is the choice of the material in which to select. Now that in many countries the old adapted " land races " have been replaced by selections, our breeding material is built up from various sources, and often the best of these are far afield. On the choice of these building stones depends the nature and range of variation. It is therefore essential that a wide range of the existing variation in any one crop plant should be available if the best choice is to be made. Breeding aims and methods alter, and hence it is highly desirable that all genic resources should be preserved for future generations. Our very efforts of producing high-yielding strains have the effect of reducing the variability of a species. This has already caused apprehension to maize workers after only a quarter of a century of intensive inbreeding.

To be of real value to the plant breeder, such collections must be observed, described and classified ; otherwise they are scarcely more useful to him than are museums or herbaria.

The first man clearly to conceive these ideas, with a rare breadth of vision, and to carry them into effect on a truly stupendous scale, was N. I. Vavilov.[33, 7] His work, though now submerged, still serves as an inspiration. His explorations and systematic observations led him to the discovery of the " centres of development," areas where cultivated plants originated and evolved and where now the greatest density of variation is to be found. These centres yielded to him not only essential knowledge, but equally essential plant material, which he brought back to the Soviet Union, and with a vast team of collaborators, grew, observed and described in a number of stations under carefully selected ecological conditions. Thus he provided the plant breeder with a great range of the world's variation, and made it accessible by devising a system of description and classification.

Outside the Soviet Union, perhaps the most successful enterprise in this field is the Commonwealth Potato Collection at Cambridge.[19] It is to be hoped that work on this great collection will be carried to its logical conclusions by developing research activities in genetics, cytology and allied fields for which it affords such unique opportunities.

An important move is the recent proposal of the Food and Agriculture Organisation of the United Nations to set up a classified catalogue of cultivated plants. All participating countries will maintain, and make available to others, all varieties which are cultivated in their area, and also breeders' lines which may be of particular interest and usefulness to breeders in other countries. Descriptions of all varieties in their national collections will be sent to F.A.O. where they will be classified and made available to breeders

all over the world. Immediate prospects are catalogues for wheat and rice, but other self-fertilised, and ultimately the cross-fertilised plants, are to follow. This scheme has many advantages. It maintains all types in their own habitats, it stimulates local interest, it is relatively inexpensive.

Its shortcomings are noted in the F.A.O. report, and it is to be hoped that they will be remedied in time. The most relevant one is that it makes no provision for the collection and classification in non-participating and especially in primitive countries, in fact, in some of those with the greatest wealth of plant types, which are still awaiting full exploration by collecting expeditions. So far the scheme fails to provide, or to offer assistance, for the establishment of world collections, with the excellent opportunity these afford for observation and research. But these gaps are mostly not final and should not detract from the real advance this scheme represents. It may be hoped that its extension to other crops will be rapid. Once it is under way much of the world's gene resources will, for the first time, be available to the whole world.

3. VARIATION AND SELECTION

In endeavouring to produce superior varieties from combinations of existing ones, the aim is either to combine one character (*e.g.* winter-hardiness) contributed by one parent, with another character (*e.g.* high yield) contributed by the other parent. Or else it may be intended to exceed the level of both parents with regard to one character such as high yield. We must now consider the variation and response to selection of the characters which determine yield.

We have already recognised the distinction between *resistance* and *production* characters. But there are further distinctions which are of relevance for an understanding of variation and selection. Some yield characters are determined by few genes with marked effects, others by a number of genes with weak individual effects [29]; Mather [8, 25] called the former *major* or *oligogenes*, the latter *polygenes*. Oligogenic characters often are not strongly influenced in their expression by the environment, they are observable even in single plants or at any rate in small families. Polygenic characters, on the other hand, are more subject to environmental variation so that their action often cannot be reliably ascertained in single plants or their immediate progenies; they are, relatively, non-observable.[10] These three kinds of distinction are not unrelated and fig. 1 illustrates the general trends of the relationships.

Resistance characters are either oligogenic (examples are stem rust and bunt in wheat, crown rust and smut in oats, wilt in flax, blight, wart and virus X in potatoes, mildew in lettuce, smudge in onions), or polygenic (*e.g.* winterhardiness, drought, lodging or shedding in wheat, woolly aphis in apples).[6, 20] More often than not

they are observable, or can be made so by modifying the environment : we infect with pathogens, use freezing chambers or drought machines ; we apply heavy fertiliser dressings to induce lodging, we seek out specific environments for comparative tests.

When the number of genes determining resistance or immunity is small, then the backcross technique, so effectively used by Briggs and his co-workers [20] in introducing resistance to bunt into Californian wheat, can be applied to great advantage. This allows the transfer of essential genes, accompanied by a minimum of the genotype of one variety, into that of another, with relatively little effort, yet with great precision. This method, which could be applied more widely than has been the case, has the great advantage of avoiding as far as is

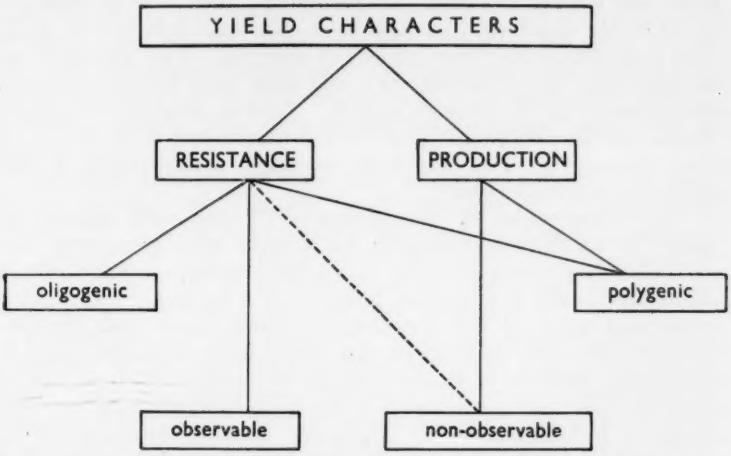

FIG. 1.—Classification of yield characters. Full lines indicate common, broken lines less common relationships.

possible the need for dealing with polygenic differences in characters other than those which are the object of resistance breeding. In other types of mating, however, this factor may impair the effect on yield which has been attained through improvement in the resistance character itself. So we see that problems of selection for polygenic characters enter into most projects of breeding for yield, though to a varying degree.

Production characters nearly always are both polygenic and non-observable. This means that breeding for such characters, as compared with resistance ones, encounters special obstacles. Genetic theory shows that the frequency with which any gene combination occurs is inversely proportional to the gene number. Hence combinations of numerous alleles can be expected only in large populations ; but if the character cannot be reliably recognised in single plants or their immediate progenies, elaborate tests are required,

the effort and expense of which reduce the size of the population to which they can be applied. So we find that, where large numbers are needed most, we can afford them least. Thus, in polygenic characters which are not observable in single plants, we are confronted with the paradox that small progress is easier to *obtain*, but great progress is easier to *find*.[10]

From this general analysis we conclude that the surest approach is the breeding for resistance characters which are—or can be made—observable. We have already seen that to remove the limitations to yield is sounder, from the viewpoint of conservation and utilisation of resources, than is the breeding for production characters proper; we now find that on the whole it is also easier. Yet progress in the latter field is both important and promising; such as is on record—*e.g.* increased sugar yield in sugar beet—has been achieved by using large populations over long periods of selection. This approach, as we have seen, has its limitations; and these have become apparent among others in the example quoted. Further progress will largely depend on our understanding of the inheritance and of the nature of yield characters. This has been strikingly demonstrated in the utilisation of hybrid vigour.

The achievements of this method [2, 20, 34]—the yield increase in maize for which it is responsible has already been mentioned—are too well known to require more than a brief reference. Self-fertilised plants are usually homozygous, hence have a polygenic system with an internal balance; but cross-fertilised plants receive their chromosome sets from two different parents, hence have a balance which is relationally selected.[25] In these plants we select individuals not so much for their own performance as for their combining ability. We select, in fact, *combinations*. These are unstable and have to be constantly reproduced. But, in the breeding of asexually propagated plants, segregation can be prevented and heterotic effects can be permanently retained by the interruption of sexual propagation. By evading segregation, we evade the restrictions on gene combination which linkage imposes, and by avoiding homozygosity we avoid the effects of harmful mutants. This method is most easily applied where fertilisation can be readily controlled (*e.g.* maize, tomatoes, pine trees) or where special controls can be introduced, such as plasmatically inherited male sterility (*e.g.* onion, sugar beet). But the potentialities of hybrid vigour are not nearly exhausted nor even fully explored (*cf.* p. 96); moreover it is a potent element in the yield increases of "polycrosses" and other "synthetic varieties" (*cf.* p. 98).

Recently Harland [18] reported on a system of repetitive selection for combining ability without previous inbreeding which he found successful in maize breeding in Peru. He proposes it as a rapid and inexpensive method for underdeveloped countries, for crops of secondary importance, or for slow-growing plants such as forest trees, coconuts, and possibly cocoa and rubber.

Other approaches for increasing the efficiency of breeding for yield have been used in recent years. The choice of parent material more often than not is empirical, so that an indication of the prospects of a cross, prior to selection, is of great advantage. Harlan *et al.*[16] found that the yields of unselected hybrid populations in F_8 served this purpose, and Immer [22] obtained similar results in F_2 or F_3.

Natural selection has been used to increase the frequency of adapted variants, both in hybrid bulks of self-fertilised plants and in populations of cross-fertilised ones. This has been done systematically in the branches of the Swedish Seed Association, but in few other places. In small countries, or for special problems, international co-operation may be called for. For example, the spreading of the combine-harvester in Western Europe has sharply accentuated the need for wheats resistant to shedding of grain. Since winds cannot be relied upon to occur each year and "synthetic" testing devices are rarely adequate, hybrid bulks—and again final observational tests—might be grown in a windy country with otherwise similar climatic conditions, such as New Zealand, the main selection being done in Europe.

Statistical methods for yield tests of large numbers have been recently devised by Yates [35, 36] and developed by Goulden and others.[12, 13] In some circumstances they have substantially increased the efficiency of tests. Much would be gained if we understood better the physiology of the interactions of heredity and environment which we are now able to measure more accurately.

Ultimately the best chance of progress comes from greater knowledge of the inheritance of yield characters and of the nature of the characters themselves. We know little of the organisation of polygenes in plants with different breeding systems, and of the progress of selection in various systems of breeding and selection. A searching enquiry into methods of selection in sugar beet is in progress at Hilleshög in Sweden, and problems of selection in wheat and other self-fertilised crops are under investigation in a number of countries, including New Zealand.

Similarly, breeding for yield would be greatly advanced by a fuller understanding of the nature of the yield characters. The breeder has received help from the plant pathologist, the analytical chemist and a variety of technologists. But an understanding of the nature and the workings of the characters we are breeding for—as distinct from their effects—still eludes us. Plant breeding institutions are by now accustomed to geneticists, pathologists, statisticians and cytologists; is it not time they opened their doors more widely to the physiologist and the biochemist?

4. NEW GENES

After more than twenty years' research it appears that induced mutation is a destructive rather than a constructive change. As

Darlington and Mather point out (p. 327), " the changes which are constructive in the sense of being new adaptations are of polygenic origin " ; " the overwhelming majority of mutants in *Drosophila* and *Antirrhinum*, especially those produced by X-rays, are hypomorphic and selectively negative, or at best selectively neutral." [8]

Well authenticated cases of useful induced mutants in crop plants and especially of mutants with increased yield, are few indeed. Gustafsson's [1, 15] extensive work, especially in barley, has shown that useful variants with specific characters (stiffness of straw, earliness) could be induced. The frequency of stiff-strawed mutants was 0·2 per cent., and one-quarter of them gave yields similar to those of the mother strain. The yield increases reported by Gustafsson are small and therefore difficult to prove.

Similar types of induced mutants have been found by Swedish and other workers in some other crop plants. It seems that X-ray (or mustard gas) induced mutability is not without promise where a specific genetic change is controlled by a change—presumably a destructive one—in a small sector of a chromosome. If this is correct, its application to plant breeding is restricted, though by no means altogether unpromising. Whether the heterotic effect of certain X-ray-induced lethal mutants could be utilised remains to be seen.[14] Lewis [23] suggests possibilities such as the production of dwarf and precocious root-stocks in cherries and pears and of self-compatible mutants, which he was able to produce in *Oenothera organensis*.

5. CHROMOSOME DOUBLING

The effect of chromosome doubling—without crossing—has been applied to plants with a variety of breeding systems. In barley, a self-fertilised plant, Gustafsson,[1] after intercrossing many tetraploid barley strains, has not got much beyond 80 per cent. of the yield of the original diploid. Among cross-fertilised plants, however, tetraploid rye and red clover [1] are distinctly promising, as is the partially triploid strain produced by Rasmusson in sugar beet. Here the tetraploid can be propagated from seed but is less productive than the diploid ; the hybrid of tetraploid and diploid produces a mixture of vigorous triploid, normal diploid and inferior tetraploid, with a composite return in excess of the diploid. In sexually propagated plants there probably is a wide field for autoploidy, the more so if the original diploid is highly heterozygous. A recent example is the tetraploid pear *Fertility* which has larger fruits than the diploid.[5]

6. SPECIES COMBINATION

The potentialities of specific and generic combinations have as yet to be fully exploited. Here there is time and need for only a few general remarks. " Wide " crosses have been made for the purpose of transferring to a cultivated species some desirable character

from a related wild or cultivated species or genus. Where there is a degree of chromosome homology, the problems of variation and selection are akin to those we have encountered in crosses within species; yet the difficulties are magnified. Where the inheritance of the desirable character and the breeding system permit a short-cut, such as recurrent backcrossing, the process is relatively simple. This is the case in the successful transference of resistance to blight and to virus X from *Solanum demissum* to our cultivated potato. In many species crosses, sterility or low crossing-over severely restrict the frequency of the desired combinations. This can be met by selecting in very large populations. Baur's [6] attempt to transfer resistance to the vine louse and mildew from the American *Vitis rupestris* to the European *Vitis vinifera*, was made with a material amounting to millions of seedlings; the transfer of resistance characters from other members of the tribe *Triticinæ*—the wheat family—to our common wheat, meets sterility barriers which have been overcome only in a few instances (*e.g.* resistance to stem rust from durum and emmer wheats). McFadden and Sears [24] recently reported an ingenious approach which appears to have great possibilities. They construct, by crossing and chromosome doubling, new species combinations containing those species from which characters are to be transferred. The new combinations, having the same chromosome number as our bread wheat, are more readily crossed with it than are the species themselves.

Species combination by crossing and chromosome doubling may be an end in itself; it results in the creation of a type with the character of a new species. While this method promises a good deal, as yet it has made no major contribution to the world's resources. Perhaps the most promising combination is the wheat-rye hybrid produced by Müntzing [1] at Svalöf. However, such new types are more or less uniform. Variability has to be *created*, just like the species itself, for without variation there is no adaptability. This can be obtained by repeating the process with various types, by further species crosses, or both. This vast effort invites co-operation; but so does also the exploitation of wide crosses. As a rule we reject in one environment what would be selected in another. On our own, we produce limited numbers of a few combinations in a restricted environment, wasting most of the proceeds; in co-operation, we can multiply the material, widen the conditions of selection, and utilise the material to the full.

7. POPULATION PROBLEMS. MAINTENANCE OF VARIETIES

From the early days of plant breeding, uniformity has been sought after with great determination. For this there are many reasons—technical, commercial, historical, psychological, æsthetic. It seems to me that the "purity concept" has not only been carried to

unnecessary lengths, but that it may be altogether inimical to the attainment of highest production.

This concept originated in the breeding of the self-fertilised cereals where high standards of homogeneity are readily attainable. But even here the extreme of purity, required by the seed regulations of various countries, is neither in itself a pre-requisite of high yield, nor is it, in fact, attainable. We know that the mutation rate is much higher than breeders often realise, and that the majority of the mutants are not distinguishable except by elaborate tests. I have maintained for years a strain of common wheat with one chromosome arm duplicated ; [11] no doubt this must have physiological effects—but it has no visible ones. " Purity " is concerned with characters which are readily seen but often are of little significance. Its excessive pursuit absorbs energies, delays progress, and deludes the breeder and the farmer as to the real merits of crop varieties. I suspect that often it is little more than a commercial convenience.

In the cross-fertilised plants, where highest purity standards are unattainable, we don't worry about it to the same extent, and no doubt this is right. We have seen that the most successful method of raising yields—the breeding for heterotic effects—succeeds through inducing the maximum of internal heterogeneity, and that internal homogeneity as a rule causes depression. " Polycrosses " [32] and other types of " synthetic varieties," such as the line mixture used by Harland [17] in cotton, achieve internal heterogeneity with simple means. But this is probably not the sole reason for their higher yields. These varieties, being biological mixtures, are genetically fluid hence they are open to improvement by selection or partial replacement ; and, being heterogeneous, they are likely to be more adaptable to variations in the environment than are rigidly uniform races. From this follows that line mixtures should give higher yields than simple pure lines, even in the self-fertilised crops, and in fact this has been found in some instances but not in others.[9]

In the cross-fertilised plants the maintenance of improved varieties is mainly concerned with retaining any heterotic effects and with excluding undesirable outcrossing, both of which are aided by reducing as far as possible the period of propagation. Yet even in this fluid material, certification systems, and price differentials, often bear little relation to the relative productivity of successive stages of multiplication, but are mainly designed with a view to administrative and commercial convenience. This is a statement of fact but not an implied criticism ; for an orderly maintenance and distribution of selected stocks is not possible without a more or less rigid system. It should be sufficiently flexible, however, to ensure a rapid distribution of superior stocks to the largest possible number of users. This cannot be achieved unless the price is within the reach of every grower.

Freedom from disease is a principal aim of varietal maintenance in all crop plants ; but this is especially so in the asexually propagated

plants which normally lack the purification through a generative phase. In such crops as potatoes, " seed " stocks are raised in areas where the carrier of virus diseases is absent, the basic nucleus being maintained in insect-proof greenhouses. One may have to go farther afield for disease-free stock : virus-free " Lloyd George " raspberries are now being sent from New Zealand to Britain. Here we find another field for international co-operation.

8. UNDERDEVELOPED CROPS : UNDERDEVELOPED COUNTRIES

In the first section of this paper I said that plant breeding had made its mark in every part of the globe. Yet, even in countries with a long tradition of plant improvement, there are crops in which nature alone has shaped heredity. Most prominent among these—though not the only ones—are the forest trees. In a report on its post-war plans [21] the Forestry Commission of Great Britain has a single reference to forest tree breeding : " Other lines of research which may be mentioned are tree breeding and vegetative reproduction ; " . . . " It is not considered that either of these two lines of research is sufficiently urgent to justify taking them up as major projects after the war." In many other countries tree breeding is not even mentioned in official reports—and this, incidentally, applies also to the programme of the Forestry Session of the present Conference. Yet, wherever it has been attacked with knowledge and vigour—mainly in Sweden, Denmark, Germany, U.S.A., Russia, South Africa and Canada [27, 31]—it shows excellent promise of large advances in yield. All the techniques we have passed in review—selection, hybrid vigour, chromosome doubling, species crosses—have been used in a variety of genera. New and old methods have been applied for speeding up flowering, fruiting and testing, with the result that breeding, especially of the shorter-lived tree types, is now relatively rapid and inexpensive.[28] Yet even were this not so, posterity has a claim to our attention.*

Some of the tropical crops have suffered similar neglect. What can be achieved is demonstrated by the great progress in crops such as sugar cane and cotton. In others much remains to be done. This applies particularly to newly-opened-up territories where new crops are introduced. It is to be hoped that schemes such as the British Food Corporation's East African Groundnut Scheme will have a team of experienced plant breeders among its research workers.

In some of the oldest countries, and crops, the position is not dissimilar. I have often wondered whether the many wheats from

* In the report of the Commission of Enquiry into the Swollen Shoot Disease of Cocoa in the Gold Coast (H.M. Stationery Office, Colonial No. 236, London, 1948), the commission finds " the development of varieties resistant to infection would be an ideal solution of the problem " but asserts that " it will be agreed that this is a long-term problem without any immediate practical significance." I, for one, cannot agree. Obviously the ideal solution of a problem which is vital to the existence of the cocoa industry is of immediate practical significance ; and a long term is not reduced by delay.

China which are more prone than any others to shed their grain, suffer severely in their own habitat ; that this is so is confirmed by Chang.[20] Even a slight improvement in the yielding capacity of rice in India or China—and the opportunities are said to be excellent [26] —would have profound effects on the nutritional standards of vast populations.

Problems such as these open wide vistas of fields in which plant breeding has as yet to make its major contribution to the resources of mankind. One sometimes feels that they dwarf the tasks on which many of us are now engaged.

9. SUMMARY

1. Plant breeding has enormously increased the world's resources by an effort and expenditure for research and for introduction into industry infinitesimal in relation to the benefits.

2. It aims at optimal adaptation of the plant to the environment—ecological, economic, social ; any change (climate, fertility, management, parasites, techniques, utilisation, tastes) demands adjustment of heredity. There can be no efficient land utilisation without efficient plant breeding.

3. Collection, survey and conservation of the world's gene resources are essential for providing breeding material for ourselves and further generations. The FAO plant catalogue should be supported, expedited and developed.

4. Breeding for resistance to factors limiting yield is usually more profitable than breeding for increased production proper ; it is easier (fewer genes, easier to observe and to test) and, with a view to resource utilisation, more economical.

5. Progress is facilitated by studies of inheritance, selection, the nature of characters—calling for co-operation of geneticists, physiologists, biochemists, pathologists, statisticians. Genetic research has yielded heterosis and backcross methods.

6. Application of induced mutation is probably limited. Chromosome doubling is promising in some cross-fertilised and asexually propagated plants.

7. Potentialities of species crosses—without and with chromosome doubling—are yet to be exploited. Resistance characters have been transferred. " Species construction " requires long-range effort, is of uncertain outcome but of ultimate promise.

8. Varietal purity can be exaggerated, it may be harmful. In cross-fertilised plants " synthetic varieties " are superior to pure stocks. Administrative and commercial considerations should be harmonised with those of production.

9. International co-operation is needed for assembling and maintaining plant collections, harnessing natural selection, organising and exploiting long-range projects, exchanging material, information and personnel.

10. Breeding of crops so far relatively neglected (especially forest trees and tropical crops), and in new or scientifically underdeveloped countries, promises a great contribution to the world's resources. Considering the world-wide shortage of trained and experienced workers, this suggests a measure of re-deployment of scientific resources.

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A POLYMERIC MULTIPLE GENE CHANGE IN HEXAPLOID WHEAT

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I. DESCRIPTION AND ORIGIN OF THE MUTANT

In an F_5 family, the progeny of an F_4 plant from the cross Tuscan × White Fife made in 1928-29, two abnormalities arose. One has already been described (Frankel, 1949*a* and *b*). It involves a series of inverted chromosome duplications which, though studied several generations later, have been traced back to the original F_5 family. The other was a chlorophyll defect—a variegation—in six of the twenty-five plants of this family. No such aberrant plants were found in any of the nine sister-families derived from the same F_4 family, nor in any other progenies from this cross, either in the same, the preceding, or the following generations. With the exception of a few green plants which by their appearance and inheritance were identified as natural crosses, the mutant strain has remained constant since it was first found in 1933-34.

Mutant plants have leaves with alternating green and white longitudinal sectors. Side tillers usually are poorer in chlorophyll than are the main tillers. When the tillers begin to elongate, the albinotic tissue turns green and is soon indistinguishable from normal. This process is typical of autumn sowings ; but when sown in the spring, or in the winter under glass, the variegation fails to develop. Following Gustafsson (1940, and personal communication) the mutant is called *striato-virescens* (*sv*). Perhaps the first case of this kind of defect was the heterozygous variegated *Vicia Faba* described by Darlington (1929). Here February-sown seedlings were green, but later turned variegated and ultimately, in their upper nodes, almost white ; but this process could be reversed by sowing in midsummer, and autumn-sown plants under glass were green.

The history of the cross in which the mutants occurred is summarised below (*g* = green, *sv* = *striato-virescens*) :

1928-29	Cross	Tuscan × White Fife	
1930-31	F_2	2800 plants	
1931-32	F_3	480 progenies at 50 plants	all *g*
1932-33	F_4	900 progenies at 50 plants	
1933-34	F_5	1599 progenies at 25 plants	
		No. 6500 : 19 *g* 6 *sv*	

Three of the *sv* plants survived to maturity ; their progenies were as follows :

No. 1934-35	*g*	*sv*
1078	0	20
1079	2	11
1082	1	12

The three green plants were doubtless natural hybrids.

Seventeen of the green plants gave progenies totalling 1440 plants ; all of these were green. One further progeny, No. 1880, consisted of 37 green and 4 *sv* plants. Of the latter, two matured and produced a total of 44 *sv* plants. The 36 green plants tested produced a total of 1212 green plants.

Thus the progeny of the F_5 plot No. 6500 shows that all plants, green as well as *sv*, were homozygous ; hence the 3 : 1 ratio is not due to monofactorial segregation.

Contamination can be excluded for two reasons. First, no other *sv*—or any other chlorophyll variants—were observed on the experimental area ; and secondly, the plant characters of the variants conformed to those of their sister plants and sister families. On the other hand contamination was probably responsible for the four *sv* plants found in the next generation in the plot No. 1880. Here also there was an absence of segregation in the progenies of the green plants ; but in this case the neighbouring plot was *sv* and hence contamination is conceivable.

2. INHERITANCE

(a) Crosses with unrelated normal

In 1934-35 the *sv* mutant was crossed reciprocally with Hunters, an unrelated variety. All F_1 plants were green. In F_2, agreement with expectation on the basis of three independent recessive genes is very close (table 1).

The analysis of a tri-factorial F_3 where the progeny size is limited requires special caution. The differentiation at the level of $P = 0{\cdot}05$ of green progenies from those segregating 63 : 1 would require a progeny size of 190 plants ; and of green and those segregating 15 : 1, one of 46 plants. Distinction between the segregation types requires the following family sizes : 234 plants to separate 63 : 1 and 15 : 1, 50 plants to separate 15 : 1 and 3 : 1—both at the levels of $P = 0{\cdot}05$, giving an actual probability of $P = 0{\cdot}025$ (Mather, 1938).

Levan (1944), using progenies of about 70 to 80 plants, grouped these according to observed frequencies of recessives and tested the resultant groupings of segregations against a variety of expectations. This procedure takes little account of the uncertainty of classification of segregating types in small families. Fröier (1946) in one F_3 eliminated altogether those families which seemed doubtful and in another grouped families by the χ^2 test.

The first problem which an array of segregating F_3 families presents is that of defining the point of separation between segregating types. This is found in the ambiguous segregation defined by Mather (1938)

TABLE 1

Segregation in F_2 of crosses striato-virescens (sv) × *green* (g)

Season	Parents	Observed		Expected		χ^2	P
		g	*sv*	*g*	*sv*		
				63 : 1		63 : 1	
1936-37	Hunters × *sv*	1050	16	*1049·3*	*16·7*	0·0263	<0·90
	sv × Hunters	1789	28	*1788·6*	*28·4*	0·0055	<0·95
1943-44	Tuscan × *sv*	3580	65	*3588·0*	*57·0*	1·1550	<0·30
1939-40	*sv* × Tuscan	340	3	*337·6*	*5·4*	1·0552	>0·30
				255 : 1		255 : 1	
1939-40	White Fife × *sv*	770	4	...	...	...	...
	sv × White Fife	512	3	...	...	...	...
	Total	1282	7	*1284·0*	*5·0*	0·7697	>0·30
1943-44	White Fife × *sv*	3048	19	*3055·0*	*12·0*	4·1289	<0·05
1948-49	*sv* × White Fife	4505	38	*4525·2*	*17·8*	23·0114	<0·001
				63 : 1		63 : 1	
				4473·0	*70·0*	14·8575	<0·001

as that ratio which will give equal χ^2's on both expectations. For the ratios 63 : 1 and 15 : 1 the ambiguous ratio is 30·74 : 1. In a progeny size of 234 the expectation for the ambiguous ratio is not more than 1 in 20 ; but in a progeny of only 45—the size available for the F_3 reported here—the expectation is as high as 1 in 5. The separation of green from 63 : 1 requires, as stated above, 190 plants and can therefore not be attempted in this material ; but the differentiation of green and 15 : 1, requiring 46 plants, and of 15 : 1 and 3 : 1 requiring 50 plants, is reasonably reliable. Moreover, to some extent misclassifications between segregation types may be expected to cancel each other out.

The grouping of F_3 progenies, using the ambiguous segregation, is given in table 2. *Striato-virescens*, the expected frequency of which is under six (*cf.* Mather, 1943) is grouped with the 3 : 1 class for the purpose of estimating χ^2.

The good fit with an expectation of three recessive genes for *striato-virescens* supports the results obtained in F_2.

(*b*) *Crosses with the parent varieties*

To test for the presence of any of the three recessive genes in the parents of the cross in which the mutant was found, homozygous *striato-virescens* plants were crossed with Tuscan and White Fife.

Again all F_1 plants, in both crosses, were green. In the Tuscan cross the F_2 segregation gave a good fit with a tri-factorial expectation (table 1). This was confirmed in an F_3 of 251 families, grouped as

TABLE 2

Frequency distribution of F_3 families in crosses striato-virescens (sv) × *green* (g)

Cross	*g*+63 : 1	15 : 1	3 : 1	*sv*	χ^2	P
sv × Hunters :—						
Observed . . .	132	32	23	1	0·8977	>0·3
Expected . . .	*132·25*	*35·25*	*20·5*			
sv × Tuscan (1944-45) :—						
Observed . . .	184	40	25	2	1·3948	<0·5
Expected . . .	*176·5*	*47·1*	*27·4*			

previously indicated (table 2). In the White Fife crosses, however, there was a deficiency of *sv*. In the early crosses the best fit was with a four-gene expectation, but more recently large F_2's gave frequencies half-way between three and four-gene expectations. Partial gametic or zygotic lethality—the most likely cause—often shows a great deal of variation in such chlorophyll defects, *e.g.* in barley (Gustafsson, 1938) and in flax (Levan, 1944).

(c) *The number of* sv *genes*

We have seen that the evidence from crosses suggests the presence of three independent recessive genes which had been acquired in the period between the cross and the F_5 in which the mutant was discovered. Is this the minimum number compatible with the evidence, or can the observed ratios be interpreted on the basis of allosyndetic pairing involving two pairs of genes?

Allosyndesis in alloploids in general and in the polyploid cereals in particular has been both inferred and observed (Winge, 1924; Darlington, 1928; Watkins and Cory, 1931; Philp, 1935). Its occurrence is liable to result in random chromosome and/or chromatid segregation, the consequences of which were recently reviewed by Little (1945). Both chromosome and chromatid segregation for less than three genes are excluded, the latter on the grounds of low frequencies of multivalents and both on the grounds of poor fit with observed ratios. For the F_2 Hunters × *striato-virescens*, *e.g.*, random chromosome segregation of two pairs of genes (35 : 1) gives $\chi^2 = 6{\cdot}435$ and $P > {\cdot}01$ (*cf.* table 1). When the F_3 is classified according to this expectation, $\chi^2 = 6{\cdot}38$ and $P < {\cdot}02$ (*cf.* table 2).

I conclude that the evidence is compatible with the assumption that the change from normal to *striato-virescens* involves three genes with independent inheritance.

3. CYTOLOGY

(a) Parents, F_1 and F_2

(i) *Univalents.*—The frequencies of univalents are given in table 3. They are of an order commonly observed in *Gramineæ* (*e.g.* Myers, 1941) and in wheat in particular (*e.g.* Hollingshead, 1932; Wylie, 1949). The high proportion of cells with four univalents, compared with those with two, indicates that possibly more than two bivalents are involved.

(ii) *Multivalents.*—The occasional occurrence of trivalents and quadrivalents is also common in wheat varieties and varietal hybrids (*cf.* Huskins, 1946; Wylie, *l.c.*). The frequencies of multivalent associations—one multivalent in 1·6 per cent. of cells in F_1 and 2·4 per cent. of cells in F_2 (table 3)—are lower than those frequently found in *Triticum vulgare* (*cf.* Wylie, *l.c.*).

TABLE 3

Frequencies of univalents and multivalents in parents, F_1 and F_2

	Number of plants	Number of cells	Frequencies of			
			2^I	4^I	$1^{III}+1^I$	1^{IV}
Tuscan	6	80	·038	...	...	...
White Fife	7	126	·048	...	·008	...
F_1	6	122	·139	·024	·008	·008
F_2	4	81	·087	·012	·012	·012

(iii) *Bridge formation.*—Anaphase bridges occur in both meiotic divisions (*cf.* Frankel, 1949*b*, table 6). Bridge frequencies in anaphase I and II respectively were 0·88 per cent. and 0·81 per cent. in F_1 and 0·75 per cent. and 0·49 per cent. in F_2. With the exception of a single second division bridge in White Fife (0·1 per cent.) no bridges were found in the parents. Of all the bridges seen, only one—a first division bridge in F_1—was accompanied by a visible fragment.

(b) The striato-virescens strain

In the mutant strain, multivalents are no more common than in the parents and in the early hybrid generations. In the many thousands of cells examined, a total of ten were found with one trivalent or quadrivalent.

The striking feature in the mutant strain is the inverted duplication (referred to in the introduction), the length of which closely approximates that of the arm to which it is attached. Purely cytological features are dealt with elsewhere (Frankel, 1949*a* and *b*); in this paper only those observations which may bear on the origin of the mutant will be discussed.

Cytological examination commenced three generations after the discovery of the mutant in an F_5 family. At this stage the inverted duplication was present throughout the mutant strain, providing evidence that the presence of the inverted duplication goes back for a minimum of three generations (Frankel, 1949*b*). At the time of examination no seed from either the original mutants or from their normal sister plants was available.

4. CORRELATION OF GENETICAL AND CYTOLOGICAL EVIDENCE

Nine of the first *striato-virescens* plants to be examined cytologically were crossed with one or the other of the original parents, Tuscan and White Fife. The mutant was the male parent in each case. This was rendered necessary by the fact that after cytological examination was completed, as a rule only one head was left which had to serve for both crossing and selfing, the mutant plants producing rarely more than two or three heads.

TABLE 4

Tuscan × striato-virescens, F_2 *segregation from cytologically identified parent and* F_1 *plants*

Parent	F_1 no.	F_2 : observed		F_2 : expected	
		g	*sv*	*g*	*sv*
(*a*) F_1 : N/N					
N/N	1297/1-7	850	14	*850·5*	*13·5*
	1304/1-5	596	9	*595·6*	*9·4*
LD/N	1299/2-4, 6, 7	594	13	*597·5*	*9·5*
	1300/1, 4, 5	360	8	*362·3*	*5·7*
LD/O	1302/3	119	2	*119·1*	*1·9*
Total	...	2519	46	*2524·9*	*40·1*
(*b*) F_1 : LD/N					
LD/N	1299/1, 5	232	6	*234·3*	*3·7*
	1300/2, 3, 6	360	3	*357·3*	*5·7*
Total	...	592	9	*591·6*	*9·4*
(*c*) F_1 : SD/N					
SD/N	1302/1, 2, 4	252	9	*256·9*	*4·1*

The *striato-virescens* parent and the F_1 plants included the following cytological types :

N/N Normal—no duplication.

LD/N heterozygote for the original (long) duplication.

LD/O 41-chromosome plant, the univalent carrying the long duplication.

SD/N heterozygote for short duplication (derived from the original duplication).

SD/O 41-chromosome plant, the univalent carrying the short duplication.

Tables 4 and 5 give the cytological classification of parents and F_1 together with the resultant F_2 segregations.

In the Tuscan crosses (table 4), the segregation type is consistent throughout, irrespective of the presence, absence or length of the duplicated segment; the segregation for *sv* is not affected by its presence or absence. Whether any of the mutant genes are located in

TABLE 5

White Fife × striato-virescens. *F_2 segregation from cytologically identified parent and F_1 plants*

Parent	F_1 no.	F_2 : observed		F_2 : expected	
		g	*sv*	*g*	*sv*
(*a*) F_1 : N/N					
N/N	1306/1-8	901	7	*904·4*	*3·6*
LD/N	1307/4, 5	217	0	*216·2*	*0·8*
	1308/6, 7	240	3	*242·1*	*0·9*
LD/O	1310/2	117	3	*119·5*	*0·5*
Total	...	1475	13	*1482·2*	*5·8*
(*b*) F_1 : LD/N					
LD/N	1307/1-3, 6	384	1	*383·5*	*1·5*
	1308/1, 3, 4, 8	484	1	*483·1*	*1·9*
LD/O	1310/3-5	355	0	*353·6*	*1·4*
Total	...	1223	2	*1220·2*	*4·8*
(*c*) F_1 : SD/N					
LD/N	1308/2, 5	246	2	*247·0*	*1·0*
LD/O	1310/1	107	2	*108·6*	*0·4*
Total	...	353	4	*355·6*	*1·4*

this pair at all can be ascertained by crossing a 41-chromosome mutant plant carrying the duplication, LD/O ♀, with normal ♂, followed by selfing—or preferably backcrossing with the triple recessive—of 41-chromosome F_1 plants. Female gametes which lack the "duplication chromosome" are viable (male gametes are not, hence the

reciprocal cross listed in table 4 could not be used); therefore 41-chromosome plants from this cross receive their only "duplication chromosome" from the normal parent, and since 40-chromosome plants are inviable the progeny will not segregate for *striato-virescens* if any of the genes are carried by this pair. This test is now under way. Incidentally a strain with a telocentric chromosome which lacks the arm carrying the duplication is *striato-virescens*; hence a mutant gene, if located in the duplication arm, must be a deletion.

When in the White Fife cross the F_2 families derived from N/N F_1 plants (table 5*a*) are compared with those from F_1 plants carrying duplications (table 5*b* and *c*), it is found that the frequency of recessives is higher in the former than in the latter. This is of doubtful significance in view of the small numbers involved, but it suggests the possibility of a connection between the inverted duplication and the differential gametic lethality suspected in this cross (*cf.* p. 106). It must be noted, however, that in the Tuscan cross the relationship is reversed.

5. DISCUSSION

I have shown in the first section that in the F_5 family in which the first mutants were found, both mutant and normal plants were homozygotes. This shows (i) that the normal plants were homozygous dominant for at least one of the three *sv* genes, and (ii) that the gene change responsible for the occurrence of the mutants took place in all probability in the somatic phase of the mother plant. We know further that both parents of the original cross were dominant for all three genes. We must now examine how the observed gene changes can be interpreted.

(*a*) *Independent mutation*

All students of chlorophyll defects in oats and wheat stress the low frequency of chlorophyll mutants. In polyploid forms, where more than one member of a polymeric series is dominant, the chance of such mutants occurring is especially low. However, they are rare even in forms where a single gene change is readily seen, *viz.* in polyploids with a single pair of dominants and in diploids.

Polymeric inheritance of genes determining chlorophyll development in the polyploid cereals is well established. Direct proof comes from the many cases in which "homologous polymeric genes" (Akerman and Fröier, 1941) have been demonstrated and from the identity of gene effects where these have been ascertained (Fröier, 1946). Indirect proof comes from comparisons of frequencies of X-ray induced chlorophyll defects in diploid and polyploid—including autoploid—forms (Stadler, 1929; Müntzing, 1941 and 1942; Fröier, 1946). Generally, the higher the degree of ploidy, the rarer the occurrence of mutants. Specifically, X-ray treatment of auto-tetraploid barley yielded no mutants in X_2, against an appreciable number in

the diploid (Müntzing, 1942). In alloploid forms, some X-ray induced chlorophyll mutants have been found in tetraploid wheat and hexaploid oats, all giving monofactorial segregation with normal ; but none were found in hexaploid wheat (Fröier, 1946). This indicates the rarity in wheat of non-duplicated genes, and of spontaneous mutation of polymeric genes.

Few spontaneous chlorophyll mutants have occurred in the cereals in controlled experiments. In the polyploid cereals they are confined to two well-established cases in hexaploid oats (Akerman, 1929 ; Akerman and Fröier, 1941 ; Fröier, 1946) and one in hexaploid wheat (Neatby, 1933). Each of these resulted from a single mutation step. In general, recessives are rare, otherwise chlorophyll defects would be found more frequently as segregates in varietal or specific crosses ; but in hexaploid oats, varieties with one and with two recessives have been found. On the whole, however, the available evidence testifies to the stability of the chlorophyll genes in the cereals in general and of the *sv* genes in particular, since the *sv* type has not been previously recorded either as a segregate or as a mutant. I conclude that the possibility of a coincidence of three independent mutations is extremely remote.

(*b*) *Gene transference through autosyndesis*

Autosyndetic pairing between homologous chromosomes in different sets of an alloploid has been established in a number of species, including the polyploid cereals (*cf.* Darlington, 1937). Watkins and Cory (1931) found genetic evidence of autosyndesis in a pentaploid wheat cross. It plays a part in the origin of shift and its breakdown (Darlington, 1928). Philp (1935) found in a progeny of a hexaploid oat cross a chlorophyll aberration which he attributed to autosyndetic pairing. He assumed that each of the parents carried one, different, recessive belonging to a polymeric series of three. Irregular disjunction as a consequence of multivalent formation—which occurs sporadically —gave rise to a 41-chromosome plant with the sole dominant gene carried by the univalent. In its progeny 40-chromosome plants are albinos, 41-chromosome plants are green. When, however, the univalent pairs autosyndetically with one of the semi-homologous chromosomes, this causes a change in the type of segregation from 1 : 6 to 3 : 1. Such a change has been observed twice in 56 progenies.

Philp assumed that two of the recessives involved were carried by the parents. In the present case, however, it is established that neither of the original parents carried any of the three genes ; hence we are forced to assume (i) that one gene mutated to the recessive allele, and (ii) that the mutant gene was transferred to the two semi-homologous chromosomes. This is a series of coincidences of normally rare events ; moreover it can scarcely be fitted into the space of time between the parent and F_5 generations.

The improbability of a gradual change arises from a further consideration. It is unlikely that a condition inducing or facilitating such a process would have been terminated with the achievement of homozygosity. That it did come to an end, however, is very likely, for had it continued, F_1 plants from crosses of mutant with normal would have yielded some F_2 families consisting solely of mutants.

(c) *Multiple mutation*

If, as we have seen, unrelated gene changes or a combination of mutation and gradual transfer are highly improbable, we are compelled to consider the possibility of related gene changes. Simultaneous gene changes in both alleles of one locus have been observed by several authors. Stubbe (1932) reported ten cases of double mutation, from the dominant to the recessive, in *Antirrhinum*; Müntzing (1937) one in *Galeopsis* from recessive to dominant; and Mather (personal communication, *cf.* also Darlington and Mather, 1949, p. 110) one in *Antirrhinum*. The specificity of multiple mutation is carried further in the present case, since both alleles of three homologues are affected.

In general, the indeterminacy of gene changes, spontaneous and induced, is as yet unchallenged: "each individual mutation remains a chance and uncontrollable event" (Muller, 1947). This mutational indeterminacy applies as a rule even where particular physiological (Muller, 1946 and 1947) or genetical (Demerec, 1937; Sturtevant, 1939; Mampell, 1943) conditions increase mutation frequencies. Yet in some specific instances the type of the resulting mutations is significantly influenced by the physiological or genetical conditions. Gustafsson (1940, 1947) found that germination onset and metabolic activity of irradiated barley seeds had specific effects on the occurrence of some types of chlorophyll mutants; this suggests a selective effect of environmental factors with regard to the nature, or at least the frequency, of mutant types. Yet the resultant mutants, though related in type, were genetically diverse. As in other mutation experiments in barley or in maize, the induced mutants were neither identical nor allelic.

Genetically controlled specificity of gene changes in *Drosophila* has been reported by Mampell (1945, 1946). A recessive mutator is responsible, in the presence of a Y-chromosome, for the production of a cytoplasmically transmitted mutator substance which induces predominantly mutants of the Minute type; but here again, while there is a degree of specificity of type, there is a large measure of genetic diversity among mutants.

In the changes to *striato-virescens* the specificity of gene change goes farther. It is an assumption, derived from the concept of polyploidy, that members of a polymeric series are identical or closely similar not only in their effects but in their constitution. Control of

such gene changes is therefore not only phenotypically or physiologically, but genetically specific.

That the duplication which was found in this mutant strain was in no way connected with the mutational process, that the joint appearance of the two phenomena in fact is a coincidence, is possible but hardly likely. It is far more probable that one is the consequence of the other ; that the unbalance caused by the cytological change destabilised elements of heredity.

(d) Polyploidy and mutation

Polyploids do not provide suitable material for a critical study of spontaneous mutation. The precision which is obtainable in diploids (*cf. e.g.* Stadler, 1946, on spontaneous mutation in maize) is barred by the multiplicity of chromosomes and genes. In one respect, however, the observations reported in this paper permit definite conclusions. It is commonly assumed (*e.g.* Huskins, 1941 ; Fröier, 1946) that in polymeric series, mutations have been accumulated over long periods of time, so that the occurrence of mutants in the various homologues may be taken as an indication of the age of a polyploid (Huskins, *l.c.*). The origin of *striato-virescens*, whatever be its cause, shows that this is not of necessity the case. Whilst there is no direct evidence that the genes involved are " homologous polymeric genes," such evidence is equally lacking in all other cases where polymeric series have been inferred. The conclusion is justified that in some circumstances parallel changes may occur, affecting more than one, and in fact all members of a polymeric series, thus breaking down the relative immunity of polyploids to the consequences of recessive mutation.

6. SUMMARY

1. A chlorophyll defect, named *striato-virescens*, which was found in an F_5 family of a varietal cross in *Triticum vulgare*, differed from both parents in three polymeric genes with independent inheritance.

2. The occurrence of the mutant coincides with that of a large inverted chromosome duplication.

3. The origin of the mutant from independent mutation in three genes, or from a single mutation transferred autosyndetically, is considered highly improbable.

4. It remains to accept the fact that a multiple mutation occurred, probably in the somatic phase. It seems that its association with the chromosome duplication is not a mere coincidence ; the duplication may be interpreted as a possible " destabiliser " of heredity.

5. The occurrence of the mutant—whatever its mode of origin—shows that recessive polymeric series may evolve suddenly, and hence not necessarily by individual steps over long spaces of time.

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THE "SEWALL WRIGHT EFFECT"

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THE current state of understanding of the Theory of Natural Selection, and the degree of appreciation which it now enjoys as a presumptive agency of evolutionary change, constitute in effect a reversal of the opinions held by the majority of geneticists during the early years of the century. This reversal followed, we believe inevitably, from the better understanding afforded by the Mendelian system of the genetic structure of natural populations, and of selection within them. It is natural enough that progress in such understanding has not always been easy, and that workers with different preconceptions have not always given equal weight to the same circumstances. The widest disparity, however, which has so far developed in the field of Population Genetics is that which separates those who accept from those who reject the theory of "drift" or "non-adaptive radiation," as it has been called by its author, Professor Sewall Wright of Chicago.

In a recent paper,[1] we criticised this theory of Sewall Wright. It claims that the subdivision of a population into small isolated or semi-isolated colonies has had important evolutionary effects; and this through the agency of random fluctuation of gene ratios, due to random reproduction in a small population.

We have long felt that there are grave objections to this view, to several of which we referred, though briefly, as it was to one of them only that our new data were directly relevant. This one, however, is completely fatal to the theory in question, namely that it is not only small isolated populations, but also large populations, that experience fluctuations in gene ratio. If this is the case, whatever other results isolation into small communities may have, any effects which flow from fluctuating variability in the gene ratios will not be confined to such subdivided species, but will be experienced also by species having continuous populations.

This fact, fatal to "The Sewall Wright Effect," appeared in our own researches from the discovery that the year-to-year changes in the gene ratio in a wild population were considerably greater than could be reasonably ascribed to random sampling, in a population of the size in question. We presumed that random sampling fluctuations must always be present, but that other causes must be acting

too, with an intensity, which, even in a population of no more than 1000, seems to be greater than the effects of random sampling. But it is only the random sampling fluctuation which is accentuated by the small size of an isolated population ; other causes, like selective survival varying from year to year, will influence large populations equally. Indeed we pointed to other researches, notably those of Dobzhansky, demonstrating such fluctuations in large populations.

This central criticism seems to have escaped Wright's attention, so that in a recent article in *Evolution* [2] he has attributed to us opinions entirely contrary to those which we hold and clearly express in our paper. Thus on p. 291 he says : " They hold that fluctuations of gene frequencies of evolutionary significance must be supposed to be due wholly to variations in selection (which they accept) or to accidents of sampling. This antithesis is to be rejected."

This passage constitutes a direct mis-statement of our published views. There is nothing in our article even to suggest the antithesis which Wright ascribes to us. Not only do we presume throughout that accidents of sampling produce their calculable effects in causing fluctuations in gene ratios, but we take some care to evaluate them. An earlier and slightly different statement by Wright to the same effect occurs on p. 281 : " Thus Fisher and Ford insist on an either-or antithesis according to which one must either hold that the fluctuations of *all* gene frequencies that are of any evolutionary significance are due to accidents of random sampling (ascribed to us), or that they are *all* due to differences in selection, which they adopt."

Nothing could be further from our actual criticism of the particular contribution to evolutionary theory which is due to Sewall Wright. He tells us that he now attaches importance to accidents of gene sampling only as one of many factors, and (p. 281) that he has always done so. This latter statement is, however, hard to reconcile with his earlier writings. Thus in the *Statistical Theory of Evolution*,[3] he says of " non-adaptive radiation " (p. 208) : " In short, this seems from statistical considerations to be the only mechanism which offers an adequate basis for a continuous and progressive evolutionary process." He ends the same paper with the sentence : " In particular, a state of sub-division of a sexually reproducing population into small, incompletely isolated groups provides the most favourable condition, not merely for branching of the species, but also for its evolution as a single group."

Sub-division into small isolated or semi-isolated populations is clearly favourable to evolutionary progress through the variety of environmental conditions to which the colonies are exposed. Moreover, so long as it could be believed that large fluctuations in gene ratios occur only in small isolated colonies by reason of fluctuations of random survival, then it *might* have been true that such fluctuations themselves favoured evolutionary change in a way that would not be allowed in a continuous distribution of the species. If now it is admitted that

large populations with continuous distributions also show year-to-year fluctuations of comparable or greater magnitude in their gene ratios, due to variable selection, the situation is entirely altered. In these circumstances, the claim for ascribing a special evolutionary advantage to small isolated communities due to fluctuations in gene ratios, had better be dropped.

Wright, and others who have supported his views, have repeatedly attempted to produce examples illustrating the spread of non-adaptive qualities. Yet the extreme difficulty of deciding what characters are of neutral survival value should be apparent : still more, the difficulty of deciding whether the total effects of the genes, or genetic situations, responsible for them are so. The fate of such speculations is well illustrated by advancing knowledge respecting the chromosome inversions found in wild populations of *Drosophila pseudo-obscura* quoted by Wright [4] p. 178, and by Sturtevant and Dobzhansky [5] as selectively neutral. Yet more recent work shows the very reverse,[6, 7, 8] and that these chromosome inversions are in fact subject to a delicate balance of selective intensity.

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A SHORT BIBLIOGRAPHY OF GENETIC RESEARCH PUBLISHED IN JAPAN, 1941-1948 †

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NOTE.—English summaries of selected papers from this list will appear in a later number of *Heredity*.

REVIEW

DICTIONARY OF GENETICS. By R. L. Knight. Waltham, Mass. : Chronica Botanica Co., and London : Dawson, 1948. Pp. xi+183. $4.50.

During its short life genetics has brought into being a great body of terminology which the newcomer to the science must seek to understand. These terms were invented to meet the needs of a specialised branch of biology, but one with which all biologists must now have some acquaintance. Though sufficiently unusual for it often to have been dubbed a jargon, genetical terminology should from this point of view present little difficulty to those accustomed, for example, to the complexities of nomenclature in Thallophytan reproductive systems or vertebrate anatomy. There is, however, a clear need for a compilation in which the meaning of genetical terms can be looked up when they are encountered for the first time.

Nor does the potential value of a genetical glossary stop at this point. The rapid growth and wide development of genetics have outstripped the codification of its terminology, at which indeed little attempt has been made. Geneticists do not always use the same terms to describe a given phenomenon, nor do they always imply the same thing by a given term. And there is little hope that any terminology could be devised which would prove acceptable to all. Indeed a case could be made out for always requiring the author of a genetical paper to include in it a selected glossary of the special terms he uses. In this way both present disparity and future evolution would be laid openly before us, instead of remaining concealed as a source of bewilderment and misunderstanding.

While, however, such a reform remains, to say the least of it, as unlikely as is general agreement on all definitions, individual attempts at compilation and codification can do much to help. Many of the terms will arouse little disagreement, and a bold attempt to provide a consistent and useful set of definitions for the remainder must aid in bringing to the surface the causes of divergent views. For these reasons the publication of a dictionary of genetical terms cannot fail to be an event of interest.

Admitting that full agreement on any set of definitions is not to be expected, by what standards can we judge of Dr Knight's success? Clearly the criteria of content, consistency and convenience must be applied, and we might expect at the same time that the author would give some indication of variation in usage where this exists.

Dr Knight's dictionary contains some 2500 terms or more. By no means all of these are, however, to be regarded as essentially genetical. Many are purely anatomical, developmental or biochemical. There may be a case for defining, for example, *vagina* and *blastomere* in a zoological text, or *anther* and *perianth* in a botanical one ; but surely the biologist interested in genetics should already be familiar with them from his earlier education. Other terms are less likely to have been encountered earlier. *Brephic* and *teleianthous*, *dystokia* and *dystrophy*, *siphonogamy* and *prometatropy* (to take a sample) will be new to many of us. But when we learn that teleianthus (of a flower) means hermaphrodite, that prometatropy means obligatory cross-pollination and that dystokia means abnormally painful and difficult parturition, we may feel that as geneticists we could well have been spared the encounter even now. There seems to be little to be gained by cluttering the book with a

mass of words which were doubtless the product of dystokia, which have never commanded wide use, at least in genetics, and which are better forgotten by geneticists. Indeed their inclusion may do active harm where the reader is a new-comer to genetics and so is in no position to recognise its legitimate terminology.

At the same time there are a few serious omissions. We have *nucleochylema* but not *nucleic acid*, *hypermorphosis* but not *hypermorph*, and *neoteinia* but not *neomorph*. Surely both the molecule which acts as midwife to all protein reproduction, and the only existing classification of mutant allelomorphs by their action deserve a better fate.

In general the definitions given are cautious : on the whole the empirical is preferred to the theoretical. Some of them can, however, hardly escape criticism. For example, in defining an *inbred line*, it is considered " as a result of inbreeding *and selection*." *Recombination* is related exclusively to crossing-over between linked genes, and its original use in relation to genes which we should now recognise as lying on different chromosomes is omitted. In defining a *gene* as a unit of inheritance no mention is made of the various means which have been used to distinguish such units one from another, the implication of the omission presumably being that the distinction can be, and is, always made by reference to recombination. We may note too that the gene is regarded as governing, affecting or controlling the transmission and development of a hereditary character, which might well suggest to the mind of the student the false rule of one gene one character. A similar lack of clear differentiation between the character and its difference is to be seen elsewhere in the book. The definition of *heterochromatin* explicitly brings in the alleged genetical property of inertness equally with the primary cytological property of stainability (which, though not so stated, is another way of saying nucleic acid charge). This term is a cytological one, and in our present state of knowledge it can be defined by reference to genetical properties only at the sacrifice of consistency as well as of authority.

From the point of view of convenience, the main criticism must be that the collection of related terms is not always carried as far as it might usefully be. Thus the definition of *chimæra* gives cross-references to seventeen other terms, nine of them being sub-classes of chimæra itself. It would be much more convenient to collect all of these nine together under sub-headings of the key word, so that the reader could compare them and see their range at a glance, rather than to leave him to turn them up one after another in widely separated parts of the book.

That Dr Knight's book will have a wide sale can hardly be doubted : the need, to meet which it was written, is too great and the market unsaturated. The nine useful appendices, including formulæ and tables, the distances recommended for avoiding contamination in seed production, and the proposed rules for gene symbolisation, must also add to the book's attraction. The author may, however, consider serious revision before any second edition, such as seems to be envisaged in the preface. In our view it would be made more compact and more useful if many of the non-genetical, out-of-the-way and obsolete terms were removed. The definitions of the remainder could then be reconsidered with a view to removing inconsistencies, achieving greater rigour and expanding them with explanatory notes where desirable.

K. MATHER

(Reviewer's italics throughout.)

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED-AND-FIRST MEETING of the Society, held on WEDNESDAY, 16th NOVEMBER 1949, at the BIOMETRY DEPARTMENT, UNIVERSITY COLLEGE, Gower Street, London, W.C. 1.

THE GENETICS OF N-TYPE SHEEP

F. W. DRY
Massey College, New Zealand
and
A. S. FRASER
Institute of Animal Genetics, Edinburgh

The *N* and *nr* genes were identified in the Romney Marsh breed, and presumably arose by mutation. Both genes condition a change in the parent breed, from the normal type which is hornless and has a longwool fleece, to the N-type, which is horned and has a carpet type of fleece. The *N* gene is a partial dominant which in $N/+$ has an expression which overlaps with the normal range. The *nr* gene is almost completely recessive. The two genes are non-allelomorphic. Preliminary analyses have been made of the genetics of modification of the expression of the *N* and *nr* genes.

"CRINKLED"—A NEW MUTANT IN THE HOUSE MOUSE, WITH DIVERSE EFFECTS ON THE SKIN AND HAIR

D. S. FALCONER, A. S. FRASER and J. W. B. KING
Institute of Animal Genetics, Edinburgh

A new mutant, which has been called "crinkled," was found in the progeny of mice treated with nitrogen mustard and has been the subject of genetical and developmental study. The mutant gene is a simple recessive and has shown no linkage with the genes *a*, *b*, c^{ch}, *d*, *p*, *s*, *bt*, *lx*, *Re*, and *sex*. The mutant phenotype shows a number of abnormalities of the skin and hair: the hairs are reduced in number and altered in structure; hairs are absent from the tail and from behind the ears: the tip of the tail is kinked: the eyelids are thickened: the ears of young mice have a peculiar shape: there is a change in the coloration of agouti animals: respiratory disorders are frequent. The majority of these abnormalities can be shown to be due to an abnormality in the development of the hair follicles, the period of development during which follicles normally form being very much shortened.

CENTROMERE PRECOCITY IN *LUZULA*

P. T. THOMAS
University College of Wales, Aberystwyth

In *Luzula campestris* and *L. maxima* (both with $2n = 12$) the chromosomes pair normally at the prophase of meiosis and the bivalents each form one to three chiasmata. Contraction due to coiling occurs during prophase, but there is delay

in attachment of nucleic acid on the chromosomes and there is also a corresponding delay in the disintegration of the nucleolus. The bivalents at diakinesis therefore appear " ghost " like although chromomeres can be clearly observed.

At metaphase the six paired configurations give the appearance of ring quadrivalents with parallel co-orientation or they could be interpreted as bivalents with diffuse centromeres (as de Camara has done for *Luzula purpurea*). But closer study has shown that the unusual appearance of the bivalents is due to a precocious division of the centromeres. Following rapid attachment of nucleic acid at the end of prophase the centromeres are seen to have divided. The bivalents " open out " into ring-like configurations in each of which 4 centromeres are co-orientated on a metaphase plate. Sometimes the separation between daughter centromeres at metaphase I. is so complete that two detached half bivalents can be observed side by side.

The consequences of this behaviour will be discussed and also the significance of these observations in relation to a possible explanation for the phenomenon of brachymeiosis in fungi.

HYBRIDS BETWEEN SOME MEMBERS OF THE RASSENKREIS *TRITURUS CRISTATUS*

H. SPURWAY

Biometry Department, University College, London

and

H. G. CALLAN

Institute of Animal Genetics, Edinburgh

The great crested newts *Triturus cristatus* are divided into various subspecies inhabitating different geographical areas. Of these we have experimented with three, namely, *T.c. carnifex*, *T.c. cristatus* and *T.c. karelinii*. Three types of hybrid F_1 have been produced by natural fertilisation : *karelinii* (female) × *cristatus* (male), *carnifex* (female) × *karelinii* (male) and *carnifex* (female) × *cristatus* (male).

In contrast to the terminal chiasma localisation which is found in the *T. vulgaris* Rassenkreis, newts of the *T. cristatus* Rassenkreis have relatively " free " distribution of chiasmata. The F_1 hybrids, however, form roughly half the chiasmata which occur in the parent races and these are terminally localised. There is frequent failure of pairing giving rise to univalents. Inversion bridges have been observed in all the hybrids and multivalent chromosome associations in hybrids involving *T.c. karelinii*, indicating that the races also differ by translocations. Spermatid degeneration occurs to varying degrees in the hybrids but all succeed in forming some mature sperm.

As might be expected having regard to the cytological evidence, the backcross and F_2 hybrids have proved to be weakly and heavy mortality has occurred during the larval and metamorphic stages.

EQUILIBRIUM UNDER NATURAL SELECTION

J. B. S. HALDANE

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In a population in equilibrium under natural selection more than one gene can only be present at a locus if (1) There is no selective effect. (2) The population forms part of a cline. (3) The selection is balanced by mutation, or (4) Heterozygotes are fitter than homozygotes. Most natural populations are very near equilibrium, and it is contended that (4) is the most important factor conserving variance. If so, natural selection determines the variances of characters as well as their means. Since homologous characters show similar coefficients of variation

in widely different species, heterosis must be biologically controlled. In random mating populations whose variance is due to heterosis fitness is correlated between sibs but not between parents and offspring, and the mean and variance of a character in the parents does not agree with that in the offspring, the parents being a group selected for phenotypes which are on the whole dominant. It is contended that these facts may explain the great slowness of evolution, and the apparent intensity of natural selection for certain characters in man, which may nevertheless be compatible with equilibrium.

THE PARTITIONING OF THE TOTAL VARIANCE IN ALL-OR-NONE CHARACTERS

A. ROBERTSON

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The analysis of all-or-none characters controlled by many pairs of genes presents some statistical difficulties. A simple analysis of variance between families as in the case of continuous variation is no longer possible. The statistical model which has to be used is that of the lexian distribution. Methods of determining the dispersion in such a distribution will be discussed and applied to data on sex-ratio.

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